Review

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Telomerase at the intersection of cancer and aging

Bruno Bernardes de Jesus and Maria A. Blasco

Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro 3, Madrid, E-28029, Spain

Although cancer and aging have been studied as independent diseases, mounting evidence suggests that cancer is an aging-associated disease and that cancer and aging share many molecular pathways. In particular, recent studies validated telomerase activation as a potential therapeutic target for age-related diseases; in addition, abnormal telomerase expression and telomerase mutations have been associated with many different types of human tumor. Here, we revisit the connection between telomerase and cancer and aging in light of recent findings supporting a role for telomerase not only in telomere elongation, but also in metabolic fitness and Wnt activation. Understanding the physiological impact of telomerase regulation is fundamental given the therapeutic strategies that are being developed that involve telomerase modulation.

Telomerase defects may lead to aging and cancer

Telomeres are repetitive DNA sequences at chromosome ends that are bound by a protective protein complex known as shelterin, which prevents them from eliciting a DNA damage response (DDR) [1,2]. Seminal studies have shown that telomeres shorten with each cell division due, in part, to the end-replication problem, that is, the inability of the DNA replication machinery to replicate DNA ends fully [3–6]. This is paralleled by the silencing of telomerase, a reverse transcriptase that is responsible for *de novo* telomere extension in most adult tissues. Some adult cell types, such as adult stem cells, have the ability to activate telomerase, particularly in the transient amplifying compartments [6]. Nevertheless, telomerase expression in stem cells is not sufficient to prevent progressive telomere shortening associated with increasing age [7].

The first connection linking telomere length to the aging process came from the observation that human primary fibroblasts had shorter telomeres with increasing donor age and that when telomeres reached a critically short length, there was a loss of proliferative ability, a terminal condition for cells known as replicative senescence [8]. It is now thought that senescence, triggered by either telomere shortening or other nontelomere-related pathways, is a key cellular outcome that may contribute to the aging process, as well as acting as a barrier for tumor progression [9]. In particular, telomere shortening and increased numbers of senescent cells have been found to occur in both proliferative and nonproliferative tissues as they age [10–12]. The importance of cellular senescence in the aging process was recently demonstrated by depletion of senescent cells in the context of an adult organism, the BubR1 progeroid mouse model, which rescued tissue dysfunction and increased organismal health span (of note, BubR1 mice present an unusually high level of senescent cells and so may not be completely reflective of the natural aging process) [13]. In a similar manner, telomerase activation strategies have been recently shown to prevent telomere shortening associated with aging, to delay organismal aging, and to increase both health span and longevity [14,15].

The anti-aging role of telomerase has been demonstrated to be largely mediated by its canonical role in elongating telomeres, which prevents the accumulation of critically short telomeres and loss of tissue homeostasis [14,15]. In particular, telomere shortening in the context of adult stem cell compartments has been previously demonstrated to cause severe impairment of stem cell mobilization and a subsequent defect in the ability to regenerate tissues [16], a situation that is similar to that of the so-called 'human telomere syndromes' [17,18]. This is because short and/or unprotected chromosome ends are recognized as persistent and/or nonrepairable DNA breaks, triggering persistent DDR [18–20], as well as cellular senescence or apoptosis mediated by the p53 pathway.

Short telomeres, and subsequent DDR activation, could occur both in cancer and aging (Figure 1). For example, an increased abundance of short telomeres correlates with higher genomic instability and decreased longevity in various organisms, including mice, zebrafish, and yeast [21–24]. In particular, mice that are deficient for telomerase or for telomere-binding proteins are characterized by accelerated age-related defects [14,16,18,19,21,22,25–32] with their telomere dysfunction load correlating with their lifespan [33]. In humans, short telomeres are considered good indicators of an individual's health status and correlate with both genetic and environmental factors [18,34–37]. Although recent findings strongly support the idea that short telomeres drive several age-related diseases [38], we cannot exclude the possibility that, in some situations, short telomeres may be a consequence of the disease itself.

Although tumors may arise from cells with short telomeres and chromosomal instability, telomerase activation and telomere maintenance are requisites for the progression of most human tumor types [39–49]. Further linking

Corresponding author: Blasco, M.A. (mblasco@cnio.es).
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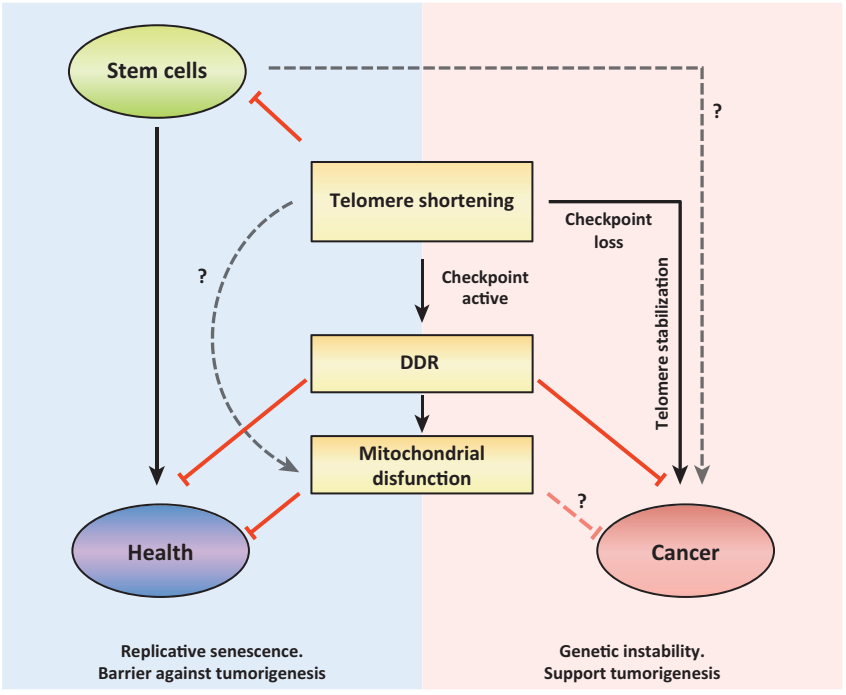

Figure 1. Short telomeres in aging and cancer. Major pathways affected by short telomeres and their impact on aging or cancer. DNA damage and tumor suppressor activity have been shown to impact tissue decline and aging. When DNA damage checkpoints are bypassed, cells with short telomeres could progress to cancer. Both the role of stem cells with short telomeres in cancer and whether short telomeres modulate other pathways independently of p53 (such as mitochondrial dysfunction) remains unknown. Abbreviation: DDR, DNA damage response.

telomerase reverse transcriptase (TERT; the human telomerase) to cancer are genome-wide association study (GWAS) results showing correlations between particular single nucleotide polymorphism (SNP) variants on the 5p15.33 bin (which includes TERT) and a higher cancer risk [21,50–58]. In particular, genetic variants in telomerase-associated genes and in the TERT-cleft lip and palate associated transmembrane protein 1 like (CLPTM1L) locus are associated with different cancer types [50,59–65]. Although the mechanism by which these variants interfere with telomerase levels and/or activity is mostly unknown, there are indications that the variants may lead to an increase in the gradual shortening of telomeres over time [52,59], but these results still need to be confirmed [66]. However, two recent studies linked melanoma risk to promoter mutations in the *TERT* gene associated with increased transcriptional activity of the TERT promoter [67,68], demonstrating the importance of tightly controlled telomerase expression.

Similar to cancer, aging encompasses a spectrum of cellular and molecular changes, but in the case of aging, these eventually result in the loss of regenerative capacity and tissue dysfunction, either through loss of functional cells or through the accumulation of surviving aberrantly damaged cells, which could result in the appearance of neoplasias. In this review, we focus on aging associated with telomere shortening, and on how telomerase could be an important therapeutic target for this process. To support the dual role of telomerase in aging and cancer, we highlight recent studies that have demonstrated that expression of telomerase in aged organisms is a valuable tool to counteract tissue degeneration through the protection of short telomeres [69], suggesting that controlled telomerase activation under particular settings delays age-related tumorigenesis.

Telomerase as a key factor that regulates aging

Evolution has developed different barriers against cancer among different species. These barriers are related to the ability to cope with DNA damage and the prevention of the accumulation of damaged cells and tissues. Irrespective of its source, damage acts as the basis for the development of dysfunctional tissues, which are a hallmark of age decline as well as the basis for cancer [69,70].

Patients carrying mutations in genes crucial for telomere maintenance show accelerated aging phenotypes. Such is the case for patients carrying mutations in *TERT*, telomerase RNA component (*TERC*) or other telomere maintenance genes, which lead to an accelerated aging syndrome known as dyskeratosis congenita (DC) [71]. DC encompasses a spectrum of pathologies, including abnormal skin pigmentation, nail dystrophy, leukoplakia, and pancytopenia [72]. In patients carrying mutations in *TERT* and *TERC*, the severity of pathologies correlates with the abundance of short telomeres; thus, the onset of disease is anticipated with increasing generations (a phenomenon known as 'genetic anticipation') [73]. Interestingly, human telomere syndromes closely recapitulate the phenotypes of previously generated mouse models for telomerase deficiency. In particular, mice genetically deficient for telomerase or some of the telomere-binding proteins present a plethora of pathologies generally characterized by the loss of tissue regeneration and organ function [32,74]. In addition to the defects in highly proliferative tissues, such as

bone marrow or skin, mice and humans with telomerase deficiency also present pathologies in more quiescent tissues, such as cardiomyopathy, insulin resistance, and lung and liver fibrosis [75,76]. To date, it remains unknown how telomerase deficiency also leads to short telomeres in tissues with a lower proliferative potential [77,78]. In this regard, mitochondrial dysfunction has been recently reported in quiescent tissues (such as the heart and liver) in the context of telomerase deficiency in mice. Several reports described that TERT that localizes at mitochondria (mt-TERT) improves mitochondrial function and protects from oxidative stress [79–81]. In particular, telomerase-deficient mice that have been bred for several generations and have an increased abundance of short telomeres present a marked mitochondrial compromise triggered by the suppression of the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha and beta (PGC1α and PGC1β networks that control, among other processes, mitochondrial function and oxidative defense [82]. Interestingly, this connection between telomere dysfunction and mitochondrial dysfunction is mediated by p53, a checkpoint common to telomere syndromes [83]. Additionally, mitochondrial dysfunction in quiescent tissues of telomerase-deficient mice could be initiated by pathways that are independent of p53 [83]. Of note, mitochondrial defects have been described in the first generation of TERT knockout (KO) mice (G1) [82], when telomere length is still conserved, demonstrating that mitochondrial dysfunction could, at least partially, precede or parallel telomere shortening. It has also been recently demonstrated that mitochondrial dysfunction is associated with physiological mouse aging, and reverted by telomerase activation [15,82].

With the aim of dissecting the role of telomerase activity and telomere length in cancer and aging, various mouse models for telomerase overexpression have been generated (Table 1). Transgenic mice that carry the mouse *TERT* gene under the control of the keratin 5 promoter (*K5-mTERT*, referred to hereafter as *TgTERT*) show increased tissue fitness; however, owing to an increased incidence of spontaneous tumors, these mice do not show an extended median lifespan [84]. To unmask the potential anti-aging role of telomerase, TgTERT mice were crossed with mice carrying extra copies of the tumor suppressors p53, p16 and Arf (Sp16/SArf/Sp53 mice), which were previously reported to be cancer resistant [14]. In this context, TgTERT/Sp16/SArf/Sp53 mice showed improved health span and a 40% increase in median longevity compared with wild type controls, or 26% compared with the long-lived and healthy Sp16/SArf/Sp53 mice, demonstrating the anti-aging activity of telomerase. A similar scenario occurred when telomerase overexpression was combined with other cancer-protective conditions, such as by subjecting mice to caloric restriction (CR). In this setting, telomerase overexpression synergized with CR to extend mouse lifespan significantly [85]. This synergy between telomerase and tumor resistance in extending organismal longevity seems to be a naturally occurring strategy, such as in the case of the mole rat or other small animals that are positive for telomerase, present higher tumor suppressor barriers [86,87] and have an unusually increased longevity for their species. Although this synergy could be a strategy in some situations, there are exceptions (such as the American beaver, another long-lived rodent, which has no detectable telomerase activity [88]), highlighting the complexity of aging.

More recently, two independent studies demonstrated that telomerase activation either in a mouse model of accelerated-aging (late-generation TERT-ER mouse model) or in natural-aged mice (1- and 2-year-old wild type mice) was sufficient to delay aging without increasing cancer incidence [15,89]. These studies support the idea that telomere shortening is one of the molecular mechanisms of cellular aging and lifespan modulation and, more notably, they demonstrate that telomerase reactivation in adult (or aged) organisms has a positive impact on delaying aging, which can be separated from its role in cancer when it is aberrantly expressed. Future work should focus on understanding the molecular mechanisms by which telomerase delays aging and disease in different organs and tissues. Below, we discuss novel pathways and telomerase partners that could be also involved in these processes.

Telomerase regulation in cancer

The role of telomerase in cancer has been extensively studied. Almost all human cancers present activation of telomerase as a hallmark, most likely as a mechanism to enable unlimited cell proliferation of tumor cells [90]. Although telomerase activation can be an early event in cancer, it is not necessary for cancer initiation [91]. However, telomerase can stimulate tumor progression by ensuring maintenance of telomeres above a critically short length, thus preventing induction of cellular senescence or apoptosis. Several mechanisms have been reported to activate telomerase in cancer, such as different oncogenes, including Myc and Wnt [92–94] that act as transcriptional regulators of telomerase. Additional telomerase activation mechanisms involving alternative splicing or epigenetic alterations have also been described [95]. Recently, mutations increasing transcriptional activity of the TERT promoter from generation of *de novo* consensus binding motifs for E-twenty-six (ETS) transcription factors have been described in human melanomas [67,68]. In addition to the canonical role of telomerase in maintaining telomeres above a critical length, telomerase has also been proposed to regulate other pathways, which could have an impact on cancer growth, such as regulation of Wnt targets and metabolism [82,96]. Getting rid of telomerase can also be problematic; the lack of telomerase could lead to increased chromosomal instability, which in turn could be the basis for cancer initiation when tumor suppressor barriers are bypassed [97]. Indeed, recent evidence demonstrated that short telomeres alone could lead to genomic instability and cancer [98]. Thus, the current view is that telomerase deficiency may contribute to the early steps of cancer development by fueling chromosomal instability, whereas subsequent activation of telomerase may be necessary to enable tumor growth and tumor progression towards more malignant states [99].

Loss-of-function and gain-of-function mouse models for telomerase have been instrumental in understanding the role of telomerase in cancer. On the one hand,

Table 1. Outcomes of enforced expression of telomerase in mice (ND – not described)

Model and/or telomerase activation	Cancer	Aging	Comments	Refs
• C57Bl/6 • Germline • K5-mTERT	Stratified epithelia histologically normal	Increased wound healing	High telomerase activity in stratified epithelia did not alter the normal epithelium structure and was not associated with changes in p53,Ras or c-Myc levels	[25]
	More tumors after DMBA+TPA treatment			
	Skin more sensitive to esters			
• FVB/n strain • Germline • CAG promoter	Higher incidence of breast carcinoma in all but one female of founder A; no differences in males	ND	No susceptibility to spontaneous or DMBA-induced papillomas in mTERT Tg mice	[129]
			Enforced mTERT expression did not alter the high rate of spontaneous tumor formation in Ink4a/Arf-deficient mice	
• C57Bl/6 • Germline • K5-mTERT and K5-mTERT/p53$^{-/-}$	Higher tumor incidence (spontaneous preneoplastic and neoplastic lesions in stratified and nonstratified epithelia)	Lower lifespan in both k5-mTERT or k5-mTERT/p53$^{-/-}$	Loss of p53 resulted in a dramatic decrease in the lifespan of these mice, concomitantly with an increased incidence of tumors, particular lymphomas	[130]
• C57Bl/6 • Germline • Lck-TERT mice	Higher incidence of spontaneous lymphoma	ND	Lck-Tert thymocytes showed greater spontaneous and IR-induced chromosomal instability	[131]
• C57Bl/6 • Germline • K5-mTERT	More hyperproliferative lesions	Increased maximal lifespan		[84]
		Decreased degenerative lesions (kidney and male germ line)		
• FVB/n strain • CMV enhancer/ β-actin promoter	ND	Enhanced hair growth through stem cell mobilization (independently of the TERC component)		[132]
• C57Bl/6 • Germline • K5-mTERT/Sp53 and K5-mTERT/Sp53/SArf/Sp16	Higher tumor incidence (mainly lymphomas) and similar lifespan (K5-mTERT/Sp53 versus K5-mTERT)	Lower tumor incidence and higher lifespan and health-span in K5-mTERT/Sp53/SArf/Sp16 versus K5-mTERT/Sp53 or WT controls		[14]
• G4$^{TERT-ER}$ mice (30–35-week-old C57Bl/6) • 4-OHT activation late in life	Telomerase activation was not sufficient to promote tumorigenesis	Extended health and lifespan	Chromosomal instability was referred	[89]
• C57Bl/6 (1- and 2-year olds) • TA-65	No increase in tumor incidence	Extended health	Activation of telomerase was not direct	[120]
		No differences in lifespan	Other studies have described similar telomerase activators in mice and humans (e.g., [127,133])	
• G4 TERT$^{-/-}$ (WW6/C57BL/6) • Ad-mTERT (specifically to the liver)	ND	Ad-mTERT injection partially rescued PGC-1α/β, Glc-6-P and Pepck expression, accompanied by a 30% increase in glucose levels relative to Ad-GFP controls, in G4-TERT$^{-/-}$ mice	Using a similar approach a previous study demonstrated restored telomerase activity and telomere function, alleviated cirrhotic pathology, and improved liver function in an experimental liver cirrhosis mouse model [134]	[82]
• C57Bl/6 (18–22 g, males and females) • Ad-mTERT-GFP (microinjection into bilateral Dg of mice)	ND	Ad-mTERT-GFP led to neurogenesis upregulation, resulted in antidepressant-like behaviors, and prevented CMS-induced behavioral modifications		[135]
• CD1 (9–11-week-old) • AGS-499	ND	Extended health (neuroprotective effects in NMDA-injected CD-1 mice)	No mechanism of telomerase activation	[128]
• C57Bl/6 (1-year and 2-year olds) • AAV9-mTERT	No increase in tumor incidence	Extended life and health span		[15]

telomerase-deficient mice (mTR$^{-/-}$) are resistant to both induced and spontaneous tumorigenesis [100], except when these mice were crossed with p53$^{+/-}$ or p53$^{-/-}$ mice [101,102]. In this scenario, a switch to epithelial carcinogenesis was observed, consistent with the role of telomere shortening in the pathophysiology of human cancers [103]. Short telomeres could be recognized as DNA double-strand (dsDNA) breaks, a deleterious DNA aberration that results in a strong activation of DDR pathways. With an intact DDR and active checkpoints, cells with dsDNA breaks activate a multitude of signaling cascades that conclude in p53 and tumor suppressor activation. This cascade of events culminates in the activation of antiproliferation signals. On the other hand, if tumor

suppressors or p53 are bypassed, a common characteristic of tumors, chromosome fusions and genomic instability could converge to give rise to cancer. This potential of telomerase to sustain the growth of tumor cells illustrates the importance of telomerase regulation in adult tissues, and probably explains why most adult cells silence telomerase expression.

Given the importance of telomerase to sustaining cancer growth, telomerase inhibitors were considered as potential therapies against tumor malignancy. However, recent evidence demonstrates that tumors in which telomerase is lost may well activate different pathways to overcome this situation, such as alternative telomere lengthening [104–106].

In addition to the canonical role of telomerase in maintaining telomeres, telomerase overexpression has also been shown to influence the regulation of the Wnt pathway, although the physiological relevance and mechanism of this regulation is still debated [15,93,94,96,107]. Nevertheless, given that telomerase activity is aberrantly overexpressed in some cancers, it is possible that Wnt modulation through higher levels of telomerase could contribute to the phenotype of some neoplasias [108].

Metabolic defects are an important link between cancer and aging. Interestingly, metabolically relevant genes that have been shown to be downregulated in the presence of short telomeres, such as *PGC1α/β*, and potentially activated by telomerase re-expression, are also linked to tumor progression [109,110]. Thus, telomerase activation in tumors may also alter cellular metabolism. Further work will be required to refine the complex relations been telomeres, telomerase, and metabolism.

In this regard, transgenic mouse models (e.g., TgTERT mice [14]) have shown that constitutive telomerase overexpression throughout mouse development results in a slightly higher incidence of cancer. Interestingly, telomerase overexpression to similar levels but in the context of the adult organism using a gene therapy strategy, showed beneficial effects, such as delayed aging and extended longevity, without increased cancer incidence [15]. This could be related to the fact that the gene-therapy vectors used [adeno-associated viruses (AAV)] led to a loss of TERT expression in highly proliferating cells or tissues. Another explanation could be that AAV preferentially targets postmitotic cells, which are potentially more resistant to cancer initiation. Alternatively, although the TgTERT mice are the product of single germline integration, they constitutively express telomerase, independently of the replicative potential of a tissue, most likely facilitating the proliferation and expansion of cells carrying pathogenic mutations.

Telomerase in stem cells

Stem cells have an important role in the aging process. Stem cell depletion seems to be at the basis of some diseases and could account for accelerated aging syndromes [111–115]. Moreover, conditions that trigger premature aging, such as telomere shortening, also impair the ability of stem cells to regenerate tissues [16]. Indeed, cells with the longest telomeres are enriched at adult stem cell niches in both mice and humans, most likely owing to the fact that these cells have the ability to activate telomerase [7,116]. However, physiological telomerase activation in stem cell compartments is not sufficient to maintain overall telomere length with aging, and telomere shortening and DNA damage accumulation is also a characteristic of aged stem cells [117].

Tumors are thought to be sustained by a subpopulation of cells with stem cell-like properties, the so-called 'cancer-initiating cells' [118,119]. It will be of interest to address whether these cancer-initiating populations also have the ability to maintain telomeres and activate telomerase activity.

Therapies based on telomerase: therapeutic value and future perspectives

As discussed above, telomerase activation is a potential therapeutic strategy for the treatment of age-related diseases [14,120]. In particular, telomerase activation in adult or old mice by means of a gene therapy strategy was shown to be sufficient to improve metabolic fitness, neuromuscular capacity, and prevent bone loss, as well as to increase significantly both median and maximum longevity, without increased cancer incidence. The finding that this strategy of telomerase activation does not lead to cancer could be due to the fact that the vectors used (AAV9) [121] are non-integrative, thus preventing the expansion of clones with telomerase overexpression [122]. Similarly, telomerase expression in an accelerated model of aging owing to telomere loss (the $G4^{TERT–ER}$ model) rescued several age phenotypes [89] and, although higher genomic instability was detected, it did not lead to an increase in tumorigenesis. These studies suggest that telomerase expression could be a feasible approach to reverse tissue dysfunction and extend healthy lifespan without increasing cancer incidence. Dedicated studies are required that use mice at different ages and comparisons at the same age, to assess the safety potential of these strategies. The actual value of these new therapies will reside in their safety, and a detailed understanding of the telomeric and nontelomeric roles of telomerase in tissue-specific healing and cancer will be crucial for considering telomerase for anti-aging therapies.

Whether these promising results could be translated to humans is unknown. It seems hazardous to use the lack of tumorigenesis in mice as evidence for the safety of pro-telomerase therapies in humans, because telomerase is differentially regulated in these organisms [123,124]. The fact that human longevity is much longer than that of mice could increase the probability of cancer formation favored by an external telomerase treatment. However, the opposing argument can be made, in that humans are more resistant to cancer than are mice and, therefore, it is less likely that telomerase activation could lead to cancer in humans compared with mice. Even though the peak of telomerase activity in humans occurs at early stages, as it does in mice, humans almost completely lose telomerase activity from somatic tissues during adulthood, contrary to mice where telomerase is found in some somatic tissues [125,126]. As a starting point for translating these findings to the clinic, telomerase activation is likely to be tested first for treatment of telomere syndromes [17]. In this scenario, the use of tissue specific gene-therapy vectors expressing

telomerase could be envisaged as a potential solution. Based on those outcomes, it will be easier to assess the feasibility of expanding telomerase activation as a strategy for combating cancer.

Concluding remarks

The finding that telomerase has roles in distinct and complementary circuitries has helped reveal its function in cancer and aging. Indeed, a change of paradigm seems to be occurring in telomerase biology, with a switch from viewing telomerase as fueling cancer to reversing aging. Telomerase expression in a background of high levels of tumor suppressors or in aged organisms seems to prevent its expected pro-cancer activity and yet it still functions as an anti-aging factor. Supporting this notion are novel telomerase activators [120,127,128], some of which are commercially available and used as anti-aging supplements. Although much of the recent work provides only proof-of-principle that telomerase works for tissue healing, we cannot dismiss the fact that, in the future, telomerase expression could be used as a safe approach for certain telomere diseases [17] or other accelerated aging syndromes.

Conflict of interest

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

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References

1 McClintock, B. (1939) The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proc. Natl. Acad. Sci. U.S.A.* 25, 405–416
2 Muller, H.J. (1938) The remaking of chromosomes. *Collecting Net* 8, 182–195
3 Hayflick, L. and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 25, 585–621
4 Levy, M.Z. *et al.* (1992) Telomere end-replication problem and cell aging. *J. Mol. Biol.* 225, 951–960
5 Olovnikov, A.M. (1996) Telomeres, telomerase, and aging: origin of the theory. *Exp. Gerontol.* 31, 443–448
6 Blasco, M.A. (2007) Telomere length, stem cells and aging. *Nat. Chem. Biol.* 3, 640–649
7 Flores, I. *et al.* (2008) The longest telomeres: a general signature of adult stem cell compartments. *Genes Dev.* 22, 654–667
8 de Jesus, B.B. and Blasco, M.A. (2012) Assessing cell and organ senescence biomarkers. *Circ. Res.* 111, 97–109
9 Collado, M. and Serrano, M. (2006) The power and the promise of oncogene-induced senescence markers. *Nat. Rev. Cancer* 6, 472–476
10 Jiang, H. *et al.* (2007) Telomere shortening and ageing. *Z. Gerontol. Geriatr.* 40, 314–324
11 Epel, E.S. *et al.* (2004) Accelerated telomere shortening in response to life stress. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17312–17315
12 Canela, A. *et al.* (2007) High-throughput telomere length quantification by FISH and its application to human population studies. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5300–5305
13 Baker, D.J. *et al.* (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236
14 Tomas-Loba, A. *et al.* (2008) Telomerase reverse transcriptase delays aging in cancer-resistant mice. *Cell* 135, 609–622
15 Bernardes de Jesus, B. *et al.* (2012) Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol. Med.* 4, 1–14
16 Flores, I. *et al.* (2005) Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* 309, 1253–1256
17 Armanios, M. and Blackburn, E.H. (2012) The telomere syndromes. *Nat. Rev. Genet.* 13, 693–704
18 Armanios, M. *et al.* (2009) Short telomeres are sufficient to cause the degenerative defects associated with aging. *Am. J. Hum. Genet.* 85, 823–832
19 Hao, L.Y. *et al.* (2005) Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. *Cell* 123, 1121–1131
20 Morrish, T.A. and Greider, C.W. (2009) Short telomeres initiate telomere recombination in primary and tumor cells. *PLoS Genet.* 5, e1000357
21 Blasco, M.A. *et al.* (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91, 25–34
22 Hemann, M.T. *et al.* (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107, 67–77
23 Lundblad, V. and Szostak, J.W. (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* 57, 633–643
24 Henriques, C.M. *et al.* (2013) Telomerase is required for zebrafish lifespan. *PLoS Genet.* 9, e1003214
25 Gonzalez-Suarez, E. *et al.* (2001) Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. *EMBO J.* 20, 2619–2630
26 Martinez, P. and Blasco, M.A. (2010) Role of shelterin in cancer and aging. *Aging Cell* 9, 653–666
27 Armanios, M.Y. *et al.* (2007) Telomerase mutations in families with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 356, 1317–1326
28 Mitchell, J.R. *et al.* (1999) A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* 402, 551–555
29 Tsakiri, K.D. *et al.* (2007) Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7552–7557
30 Vulliamy, T. *et al.* (2001) The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* 413, 432–435
31 Yamaguchi, H. *et al.* (2005) Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N. Engl. J. Med.* 352, 1413–1424
32 Martinez, P. and Blasco, M.A. (2011) Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins. *Nat. Rev. Cancer* 11, 161–176
33 Vera, E. *et al.* (2012) The rate of increase of short telomeres predicts longevity in mammals. *Cell Rep.* 2, 732–737
34 Alder, J.K. *et al.* (2008) Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13051–13056
35 Benetos, A. *et al.* (2004) Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. *Hypertension* 43, 182–185
36 Cipriano, C. *et al.* (2009) Accumulation of cells with short telomeres is associated with impaired zinc homeostasis and inflammation in old hypertensive participants. *J. Gerontol. A: Biol. Sci. Med. Sci.* 64, 745–751
37 Elvsashagen, T. *et al.* (2011) The load of short telomeres is increased and associated with lifetime number of depressive episodes in bipolar II disorder. *J. Affect. Disord.* 135, 43–50
38 Codd, V. *et al.* (2013) Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* 45, 422–427 427e421–422
39 Chadeneau, C. *et al.* (1995) Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Res.* 55, 2533–2536
40 Tang, R. *et al.* (1998) Close correlation between telomerase expression and adenomatous polyp progression in multistep colorectal carcinogenesis. *Cancer Res.* 58, 4052–4054
41 Fang, D.C. *et al.* (2001) Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer. *World J. Gastroenterol.* 7, 522–526
42 Engelhardt, M. *et al.* (1997) Telomerase and telomere length in the development and progression of premalignant lesions to colorectal cancer. *Clin. Cancer Res.* 3, 1931–1941
43 Maruyama, Y. *et al.* (1997) Telomere length and telomerase activity in carcinogenesis of the stomach. *Jpn. J. Clin. Oncol.* 27, 216–220

44 Lantuejoul, S. *et al.* (2005) Telomere shortening and telomerase reverse transcriptase expression in preinvasive bronchial lesions. *Clin. Cancer Res.* 11, 2074–2082
45 Meeker, A.K. *et al.* (2004) Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. *Am. J. Pathol.* 164, 925–935
46 van Heek, N.T. *et al.* (2002) Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am. J. Pathol.* 161, 1541–1547
47 Meeker, A.K. *et al.* (2002) Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res.* 62, 6405–6409
48 Finley, J.C. *et al.* (2006) Chromosomal instability in Barrett's esophagus is related to telomere shortening. *Cancer Epidemiol. Biomarkers Prev.* 15, 1451–1457
49 Zheng, Y.L. *et al.* (2009) Telomere attrition in cancer cells and telomere length in tumor stroma cells predict chromosome instability in esophageal squamous cell carcinoma: a genome-wide analysis. *Cancer Res.* 69, 1604–1614
50 McKay, J.D. *et al.* (2008) Lung cancer susceptibility locus at 5p15.33. *Nat. Genet.* 40, 1404–1406
51 Wang, Y. *et al.* (2008) Common 5p15.33 and 6p21.33 variants influence lung cancer risk. *Nat. Genet.* 40, 1407–1409
52 Hills, M. and Lansdorp, P.M. (2009) Short telomeres resulting from heritable mutations in the telomerase reverse transcriptase gene predispose for a variety of malignancies. *Ann. N. Y. Acad. Sci.* 1176, 178–190
53 Willeit, P. *et al.* (2010) Telomere length and risk of incident cancer and cancer mortality. *JAMA* 304, 69–75
54 Londono-Vallejo, J.A. (2004) Telomere length heterogeneity and chromosome instability. *Cancer Lett.* 212, 135–144
55 Meeker, A.K. *et al.* (2004) Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin. Cancer Res.* 10, 3317–3326
56 Calado, R.T. and Chen, J. (2006) Telomerase: not just for the elongation of telomeres. *Bioessays* 28, 109–112
57 Hosgood, H.D., III *et al.* (2009) Genetic variation in telomere maintenance genes, telomere length, and lung cancer susceptibility. *Lung Cancer* 66, 157–161
58 Van Dyke, A.L. *et al.* (2009) Chromosome 5p region SNPs are associated with risk of NSCLC among women. *J. Cancer Epidemiol.* 2009, 242151
59 Rafnar, T. *et al.* (2009) Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nat. Genet.* 41, 221–227
60 Shete, S. *et al.* (2009) Genome-wide association study identifies five susceptibility loci for glioma. *Nat. Genet.* 41, 899–904
61 Wrensch, M. *et al.* (2009) Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nat. Genet.* 41, 905–908
62 Jin, G. *et al.* (2009) Common genetic variants on 5p15.33 contribute to risk of lung adenocarcinoma in a Chinese population. *Carcinogenesis* 30, 987–990
63 Landi, M.T. *et al.* (2009) A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. *Am. J. Hum. Genet.* 85, 679–691
64 Petersen, G.M. *et al.* (2010) A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. *Nat. Genet.* 42, 224–228
65 Hofer, P. *et al.* (2011) MNS16A tandem repeats minisatellite of human telomerase gene: a risk factor for colorectal cancer. *Carcinogenesis* 32, 866–871
66 Pooley, K.A. *et al.* (2010) No association between TERT-CLPTM1L single nucleotide polymorphism rs401681 and mean telomere length or cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 19, 1862–1865
67 Huang, F.W. *et al.* (2013) Highly recurrent TERT promoter mutations in human melanoma. *Science* 339, 957–959
68 Horn, S. *et al.* (2013) TERT promoter mutations in familial and sporadic melanoma. *Science* 339, 959–961
69 de Jesus, B.B. and Blasco, M.A. (2012) Potential of telomerase activation in extending health span and longevity. *Curr. Opin. Cell Biol.* 24, 739–743
70 Serrano, M. and Blasco, M.A. (2007) Cancer and ageing: convergent and divergent mechanisms. *Nat. Rev. Mol. Cell Biol.* 8, 715–722
71 Calado, R.T. and Young, N.S. (2009) Telomere diseases. *N. Engl. J. Med.* 361, 2353–2365
72 Dokal, I. (2011) Dyskeratosis congenita. *Hematology Am. Soc. Hematol. Educ. Program* 2011, 480–486
73 Armanios, M. (2009) Syndromes of telomere shortening. *Annu. Rev. Genomics Hum. Genet.* 10, 45–61
74 Beier, F. *et al.* (2012) Conditional TRF1 knockout in the hematopoietic compartment leads to bone marrow failure and recapitulates clinical features of Dyskeratosis congenita. *Blood* 120, 2990–3000
75 Leri, A. *et al.* (2003) Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J.* 22, 131–139
76 Basel-Vanagaite, L. *et al.* (2008) Expanding the clinical phenotype of autosomal dominant dyskeratosis congenita caused by TERT mutations. *Haematologica* 93, 943–944
77 Aikata, H. *et al.* (2000) Telomere reduction in human liver tissues with age and chronic inflammation. *Exp. Cell Res.* 256, 578–582
78 Chimenti, C. *et al.* (2003) Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ. Res.* 93, 604–613
79 Chiodi, I. and Mondello, C. (2012) Telomere-independent functions of telomerase in nuclei, cytoplasm, and mitochondria. *Front. Oncol.* 2, 133
80 Saretzki, G. (2009) Telomerase, mitochondria and oxidative stress. *Exp. Gerontol.* 44, 485–492
81 Gordon, D.M. and Santos, J.H. (2010) The emerging role of telomerase reverse transcriptase in mitochondrial DNA metabolism. *J. Nucleic Acids* http://dx.doi.org/10.4061/2010/390791
82 Sahin, E. *et al.* (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 470, 359–365
83 Sahin, E. and DePinho, R.A. (2012) Axis of ageing: telomeres, p53 and mitochondria. *Nat. Rev. Mol. Cell Biol.* 13, 397–404
84 Gonzalez-Suarez, E. *et al.* (2005) Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice. *Oncogene* 24, 2256–2270
85 Vera, E. *et al.* (2013) Telomerase reverse transcriptase synergizes with calorie restriction to increase health span and extend mouse longevity. *PLoS ONE* 8, e53760
86 Gorbunova, V. and Seluanov, A. (2009) Coevolution of telomerase activity and body mass in mammals: from mice to beavers. *Mech. Ageing Dev.* 130, 3–9
87 Seluanov, A. *et al.* (2009) Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19352–19357
88 Gorbunova, V. *et al.* (2008) Rodents for comparative aging studies: from mice to beavers. *Age* 30, 111–119
89 Jaskelioff, M. *et al.* (2011) Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* 469, 102–106
90 Shay, J.W. and Bacchetti, S. (1997) A survey of telomerase activity in human cancer. *Eur. J. Cancer* 33, 787–791
91 Hackett, J.A. and Greider, C.W. (2002) Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. *Oncogene* 21, 619–626
92 Wu, K.J. *et al.* (1999) Direct activation of TERT transcription by c-MYC. *Nat. Genet.* 21, 220–224
93 Hoffmeyer, K. *et al.* (2012) Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 336, 1549–1554
94 Greider, C.W. (2012) Molecular biology. Wnt regulates TERT: putting the horse before the cart. *Science* 336, 1519–1520
95 Kyo, S. and Inoue, M. (2002) Complex regulatory mechanisms of telomerase activity in normal and cancer cells: how can we apply them for cancer therapy? *Oncogene* 21, 688–697
96 Park, J.I. *et al.* (2009) Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature* 460, 66–72
97 Feldser, D.M. and Greider, C.W. (2007) Short telomeres limit tumor progression in vivo by inducing senescence. *Cancer Cell* 11, 461–469
98 Begus-Nahrmann, Y. *et al.* (2012) Transient telomere dysfunction induces chromosomal instability and promotes carcinogenesis. *J. Clin. Invest.* 122, 2283–2288
99 Ding, Z. *et al.* (2012) Telomerase reactivation following telomere dysfunction yields murine prostate tumors with bone metastases. *Cell* 148, 896–907
100 Gonzalez-Suarez, E. *et al.* (2000) Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat. Genet.* 26, 114–117

101 Chin, L. *et al.* (1999) p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* 97, 527–538
102 Artandi, S.E. *et al.* (2000) Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* 406, 641–645
103 Chang, S. *et al.* (2001) Modeling chromosomal instability and epithelial carcinogenesis in the telomerase-deficient mouse. *Semin. Cancer Biol.* 11, 227–239
104 Hu, J. *et al.* (2012) Antitelomerase therapy provokes ALT and mitochondrial adaptive mechanisms in cancer. *Cell* 148, 651–663
105 Sachsinger, J. *et al.* (2001) Telomerase inhibition in RenCa, a murine tumor cell line with short telomeres, by overexpression of a dominant negative mTERT mutant, reveals fundamental differences in telomerase regulation between human and murine cells. *Cancer Res.* 61, 5580–5586
106 Herrera, E. *et al.* (2000) Impaired germinal center reaction in mice with short telomeres. *EMBO J.* 19, 472–481
107 Strong, M.A. *et al.* (2011) Phenotypes in mTERT+/- and mTERT-/- mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase. *Mol. Cell. Biol.* 31, 2369–2379
108 Liang, Y. *et al.* (2010) Stem-like cancer cells are inducible by increasing genomic instability in cancer cells. *J. Biol. Chem.* 285, 4931–4940
109 Zhu, Y. *et al.* (1999) Amplification and overexpression of peroxisome proliferator-activated receptor binding protein (PBP/PPARBP) gene in breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10848–10853
110 Bhalla, K. *et al.* (2011) PGC1alpha promotes tumor growth by inducing gene expression programs supporting lipogenesis. *Cancer Res.* 71, 6888–6898
111 Rosengardten, Y. *et al.* (2011) Stem cell depletion in Hutchinson–Gilford progeria syndrome. *Aging Cell* 10, 1011–1020
112 Schlessinger, D. and Van Zant, G. (2001) Does functional depletion of stem cells drive aging? *Mech. Ageing Dev.* 122, 1537–1553
113 Ruzankina, Y. *et al.* (2007) Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. *Cell Stem Cell* 1, 113–126
114 Rossi, D.J. *et al.* (2007) Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729
115 Rando, T.A. (2006) Stem cells, ageing and the quest for immortality. *Nature* 441, 1080–1086
116 Jung, P. *et al.* (2011) Isolation and in vitro expansion of human colonic stem cells. *Nat. Med.* 17, 1225–1227
117 Sperka, T. *et al.* (2012) DNA damage checkpoints in stem cells, ageing and cancer. *Nat. Rev. Mol. Cell Biol.* 13, 579–590
118 Jordan, C.T. *et al.* (2006) Cancer stem cells. *N. Engl. J. Med.* 355, 1253–1261
119 Reya, T. *et al.* (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414, 105–111
120 de Jesus, B.B. *et al.* (2011) The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging Cell* 10, 604–621
121 Kaplitt, M.G. *et al.* (2007) Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 369, 2097–2105
122 Buning, H. *et al.* (2008) Recent developments in adeno-associated virus vector technology. *J. Gene Med.* 10, 717–733
123 Fujiki, T. *et al.* (2010) Regulatory mechanisms of human and mouse telomerase reverse transcriptase gene transcription: distinct dependency on c-Myc. *Cytotechnology* 62, 333–339
124 Weise, J.M. and Gunes, C. (2009) Differential regulation of human and mouse telomerase reverse transcriptase (TERT) promoter activity during testis development. *Mol. Reprod. Dev.* 76, 309–317
125 Collins, K. and Mitchell, J.R. (2002) Telomerase in the human organism. *Oncogene* 21, 564–579
126 Prowse, K.R. and Greider, C.W. (1995) Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl. Acad. Sci. U.S.A.* 92, 4818–4822
127 Harley, C.B. *et al.* (2011) A natural product telomerase activator as part of a health maintenance program. *Rejuvenation Res.* 14, 45–56
128 Eitan, E. *et al.* (2012) Novel telomerase-increasing compound in mouse brain delays the onset of amyotrophic lateral sclerosis. *EMBO Mol. Med.* 4, 313–329
129 Artandi, S.E. *et al.* (2002) Constitutive telomerase expression promotes mammary carcinomas in aging mice. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8191–8196
130 Gonzalez-Suarez, E. *et al.* (2002) Cooperation between p53 mutation and high telomerase transgenic expression in spontaneous cancer development. *Mol. Cell. Biol.* 22, 7291–7301
131 Canela, A. *et al.* (2004) Constitutive expression of tert in thymocytes leads to increased incidence and dissemination of T-cell lymphoma in Lck-Tert mice. *Mol. Cell. Biol.* 24, 4275–4293
132 Sarin, K.Y. *et al.* (2005) Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* 436, 1048–1052
133 Fauce, S.R. *et al.* (2008) Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *J. Immunol.* 181, 7400–7406
134 Rudolph, K.L. *et al.* (2000) Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science* 287, 1253–1258
135 Zhou, Q.G. *et al.* (2011) Hippocampal telomerase is involved in the modulation of depressive behaviors. *J. Neurosci.* 31, 12258–12269