Review

Mark A. Babizhayev*, Khava S. Vishnyakova and Yegor E. Yegorov

Hormone-brain-aging relationships, broadly reactive with imidazole-containing dipeptides: targeting of telomere attrition as an aging biomarker and dynamic telomerase activity flirting

Abstract: It has been documented that telomere-associated cellular senescence may contribute to certain age-related disorders, and telomere length (TL) may be an informative biomarker of healthy aging. Hormone-brain-aging behavior-modulated telomere dynamics and changes in telomerase activity are consistent elements of cellular alterations associated with changes in proliferative state, and these processes are consequently considered as the new therapeutic drug targets for physiological control with advanced drug delivery and nutritional formulations. We raise and support a therapeutic concept of using nonhydrolyzed forms of naturally occurring neuron-specific imidazole dipeptide-based compounds carnosine and carcinine, making it clinically possible that slowing down the rate of telomere shortening could slow down the human aging process in specific tissues where proliferative senescence is known to occur, with the demonstrated evidence of telomere shortening that appeared to be a hallmark of oxidative stress and disease. Carnosine released from skeletal muscle during exercise may be transported into the hypothalamic tuberomammillary nucleus (TMN) histamine neurons and hydrolyzed. The resulting L-histidine may subsequently be converted into histamine, which could be responsible for the effects of carnosine on neurotransmission and hormone-like antiaging physiological function. The preliminary longitudinal studies of elderly individuals suggest that longer telomeres are associated with better survival, and an advanced oral nutritional support with nonhydrolyzed carnosine (or carcinine and patented compositions thereof) is a useful therapeutic tool for a critical TL maintenance that may fundamentally be applied in the treatment of age-related sight-threatening eye disorders, prolonged life expectancy, increased survival and chronological age of an organism in health control, smoking behavior, and disease.

“Our pleasures were simple—they included survival.”
— Dwight D. Eisenhower, 34th President of the United States, 1953–1961

Keywords: age-related diseases; carcinine, N-acetylcarnosine, nonhydrolyzed carnosine; cumulative oxidative stress; healthy aging; hormone-brain-aging behavior relationships; natural imidazole-containing peptidomimetics; pharmaconutritional support; telomere length protection; telomeres and telomerase biology.

DOI 10.1515/jbcpp-2014-0045
Received April 8, 2014; accepted May 10, 2014; previously published online August 13, 2014

Introduction

Biomarkers allow a better measurement of health and disease and a corroboration of self-reported health behaviors and indicators of exposures to environmental agents. Using biomarkers to identify individual differences in exposure to environmental risk factors, health, or physical activity level can improve the understanding of how these factors vary with life histories and their long-term health consequences and survival.
Aging is considered eventually as a near universal process, yet the molecular mechanisms that underlie cellular senescence have remained elusive. Understanding the aging process is central to preventing age-related disease burden and premature mortality with therapeutic strategies. Many different measures have been suggested as having prognostic value for mortality [1, 2]. Such knowledge may be directly translated into behavioral or treatment recommendations that improve health among the elderly [2]. It occurred that, compared to the detailed information we have about many biological processes, we know surprisingly little about what determines the life span for the protection of human subjects and animals in research.

Telomeres are biomarkers of healthy aging, disease, and exposure of environmental risk factors that have prognostic value for drug treatments

Cellular aging may offer insights into organismal aging relevant to chronic diseases such as cardiovascular disease (CVD) [1]. It is thought that a limited investment by the body in many types of maintenance and repair causes aging. Cell turnover is one mechanism of replacing damaged cells, and cell division thus contributes to good repair, but the number of times cells can divide is limited to form a barrier against cancer.

Telomeres, the protective nucleoprotein structures capping the ends of eukaryotic chromosomes, can serve a portion of the telomere cap that is not replicated due to the “end replication problem”; that is, DNA polymerase does not completely replicate the end of a DNA strand [3]. Telomeres are dynamic DNA-protein structures and are essential for chromosomal stability and cell viability in different species. Hence, cells in certain older organisms, including humans, have shorter telomeres on average than cells in younger organisms. Telomere length (TL) has been proposed as a marker of mitotic cell age and as a general index of human organismal aging. Telomere shortening is a feature of cellular aging common to a range of human tissues. It has been suggested that TL plays a crucial role in tissue functioning and, by extension, in the life expectancy of whole organisms [1]. Short absolute leukocyte TL has been linked to cardiovascular-related morbidity and mortality (for detailed analysis, see “Clinical disorders associated with age-dependent telomere loss: telomerase-based biological strategies” [1]. Telomere shortening accompanies human aging, and premature aging syndromes are often associated with short telomeres. These two observations are central to the hypothesis that TL directly influences longevity [4]. If true, genetically determined mechanisms of TL homeostasis should significantly contribute to variations of longevity in the human population. On the contrary, telomere shortening is also observed in the course of many aging-associated disorders [4]. Recently, it has been proposed that TL may not be a strong biomarker of survival in older individuals, but it may be rather an informative biomarker of healthy aging [5]. Eventually, once better controls and therapeutic treatments for aging and age-related disorders are available, cellular rejuvenation by manipulating telomeres and enzyme telomerase activity may reduce some of the physiological declines that accompany aging. Such treatments should increase health span, but because replicative aging represents only one of many processes that may contribute to overall human aging, modest increases in life span might be expected at best [6].

The mechanisms regulating organismal aging are complex, and it is unlikely that any single mechanism will explain all or even most of the molecular and physiological changes that occur as we age. However, in this work, we raise a new therapeutic concept, making it clinically possible that slowing down the rate of telomere shortening could slow down the human aging process in specific tissues where proliferative senescence is known to occur, with the demonstrated evidence of telomere shortening appeared to be a hallmark of oxidative stress and disease [7–10].

Controls for the length of life, survival, and chronological age

One of the greatest scientific mysteries, which have puzzled scientists for thousands of years, is what controls the length of life (reviewed in ref. [11]). At a time when human life span is increasing to previously unforeseen lengths, it is urgent that this ancient question is answered. Fortunately, recent progress suggests that the answers will soon be at hand. Animal species exhibit great diversity in their maximum life span [12]. Because length of life is determined by survival, and survival is a trait on which Darwinian natural selection is expected to act with particular force, there is strong interest in trying to understand the factors that control the length of life. There is also a biomedical and social urgency in doing so as soon as possible, in view of the remarkable increases in human life expectancy in recent decades.
Figure 1  N-acetylcarnosine lubricant eye drops as a therapeutic tool and powerful platform to manage human cataracts. (A) Tissue damage due to oxidative stress has been implicated in aging, memory loss, and cataract formation as a biomarker of human senescence. The age-specific rates of age-related cataract for a group of 100 (50 males, 50 females) elderly persons who reached at least age 90 years with preserved cognition were compared to the corresponding rates of age-related cataract reported in five population-based studies. The principal finding was that persons who achieved exceptional longevity with preserved cognition (successful aging) group manifested a significant reduction in the age-specific rate and lifetime cumulative incidence of age-related cataract compared to the general population [13]. The findings suggest that the progressive development of lens opacities may be reflective of degenerative events occurring more generally throughout the body [13]. Carnosine, released ophthalmically from N-acetylcarnosine prodrug lubricant eye drops, at physiological concentration might remarkably reduce the rate of telomere shortening in the lens cells subjected to oxidative stress in the lack of efficient antioxidant lens protection (B). The data of visual functions (visual acuity, glare sensitivity) in older adult subjects and older subjects with cataract treated with 1% N-acetylcarnosine lubricant eye drops showed significant improvement compared, in contrast to the control group that showed generally no improvement in visual functions, with no difference from baseline in visual acuity and glare sensitivity readings [14, 15].

The age of a deteriorating object is described by the corresponding process of degradation and is compared with the chronological age. According to numerous theories (reviewed in refs. [17, 18]), the nature of biological aging is in some “wearing” (damage accumulation). For instance, damage accumulation can be due to nonideal repair mechanisms or (and) accumulation of deleterious mutations. The logic of the disposable soma theory tells us that there are likely to be multiple kinds of damage that contribute to aging, which are regulated by a complex network of maintenance and repair functions. It follows that aging is not itself genetically programmed, although longevity is, through the genetic regulation of repair mechanisms. It also follows that multiple molecular mechanisms are expected to participate in cell aging and that the actions of these mechanisms are inherently stochastic, that is, subject to the variations of chance. It also tells us that the mechanisms of cellular and molecular aging are inherently stochastic – strongly influenced by chance – which may explain the marked variability in life span even among genetically identical populations. One possible mechanism by which increased DNA damage could lead to cellular degeneration and death is by stochastic deregulation of gene expression. These results underscore the stochastic nature of the aging process and could provide a mechanism for age-related cellular degeneration and death in tissues of multicellular organisms.
Aging implies a decline in life expectancy and stress-coping ability and increased prevalence of comorbidity, increased risk functional dependence, and of the need of social support [20]. Although it is universal, aging is highly individualized and is poorly reflected in chronological age. The management of the older of aged person should be based on an assessment of physiological rather than chronological age [20].

Central to the current understanding of biological aging is the idea that limited evolutionary investment in mechanisms of cellular and molecular maintenance and repair causes a gradual accumulation of cellular damage, which in turn causes age-related frailty, disease, and death [11]. If we want to understand the underlying mechanisms satisfactorily, the inherent complexity of aging cannot be ignored and reductionist science alone will not be enough. Much of the early, bewildering proliferation of aging theories during the 1950s, 1960s, and 1970s arose from a tendency to view the different aging hypotheses as competing (reviewed in ref. [11]). However, the disposable soma theory suggests that multiple kinds of damage will accumulate in parallel within cells. This has led to recent initiatives to develop “network” theories of aging in which the contributions of various mechanisms are considered together, thereby allowing for interaction and synergism between different processes [16].

The essential multiplicity of mechanisms of aging is widely acknowledged, although in practice it has not yet been much studied, whereas the stochastic nature of aging is evident in the pronounced variability (e.g., heterogeneity between organisms, cell types, and clonal populations, even in identical environments) that is a hallmark of the aging process [19–23]. The replicative life span of primary human cells is telomere dependent; however, its heterogeneity is not understood.

It is natural to apply to organisms some therapeutic concepts and approaches used to increase life expectancy, survival, and chronological age in degrading (aging) living organisms and the human body biological systems. The aging of organisms is characterized by a gradual functional decline of all organ systems. Mammalian somatic cells in culture display a limited proliferative life span, at the end of which they undergo an irreversible cell cycle arrest known as replicative senescence. Whether cellular senescence contributes to organismal aging has been controversial. Cellular aging may offer insights into organismal aging relevant to chronic diseases such as cardiovascular disease (CVD).

In this review article, we investigate telomere dysfunction/shortening, a recently discovered biomarker of cellular senescence. The question of how and under what circumstances telomere dynamics and replicative senescence are tied to organismal life span is still unsolved (Figure 2) [23, 24]. We discuss various molecular mechanisms that are likely to cause the observed specific telomere dynamics, including cell division, oxidative stress, and telomerase activity, and the effects of imidazole-containing dipeptide-based compounds on these parameters of cellular function regulation, organismal life span, and survival.

**Molecular biologics of telomere-dependent senescence**

The terminus of a DNA helix has been called its Achilles’ heel. Thus, to prevent possible incomplete replication and instability of the termini of linear DNA, eukaryotic chromosomes end in characteristic repetitive DNA sequences within tailor-made structures called telomeres. In immortal cells, loss of telomeric DNA due to degradation or incomplete replication is apparently balanced by telomere elongation, which may involve de novo synthesis of additional repeats by novel DNA polymerase called telomerase [25]. Telomeres are specialized structures at the ends of eukaryotic linear chromosomes, consisting of protein-bound tandemly repeated simple DNA sequences [26]. Recent works have highlighted its remarkable mode of synthesis by the ribonucleoprotein reverse transcriptase, telomerase, as well as its ability to form unusual space filling structures (reviewed in ref. [27]). As can be seen in Figure 3A–C, telomerase is a ribonucleoprotein polymerase that specifically elongates telomeres. The telomeric DNA is unique in that it is copied from an RNA template that forms part of the enzyme telomerase (Figure 3C). The DNA of telomeres, being the terminal DNA-protein complexes of chromosomes, differs notably from other DNA sequences in both structure and function. In response to DNA damage, chromatin undergoes a global decondensation process that has been proposed to facilitate genome surveillance. The slight differences in the epigenetic configuration might account for the cell-to-cell variation in the strength of the DNA damage response observed when groups of cells are challenged with DNA breaks [32]. Telomere synthesis by telomerase has been shown to be essential for telomere structures maintenance, survival, and long-term viability [27].

Telomeres in most human cells shorten with each round of DNA replication, because they lack the enzyme telomerase. This is not, however, the only determinant
of the rate of loss of telomeric DNA. Oxidative damage is repaired less well in telomeric DNA than elsewhere in the chromosome, and oxidative stress accelerates telomere loss, whereas antioxidants decelerate it. It was suggested that oxidative stress is an important modulator of telomere loss and that telomere-driven replicative senescence is primarily a stress response (Figure 3B) [33].

Most human somatic cells can undergo only a limited number of population doublings (PD) in vitro. This exhaustion of proliferative potential, called senescence, can be triggered when telomeres cannot fulfill their normal protective functions. The telomere-initiated senescence reflects a DNA damage checkpoint response that is activated with a direct contribution from dysfunctional telomeres. The senescent human fibroblasts display molecular markers characteristic of cells bearing DNA double-stranded breaks [34]. These markers include nuclear foci of phosphorylated histone H2AX and their colocalization with DNA repair and DNA damage checkpoint factors such as 53BP1, MDC1, and NBS1. The senescent cells contain activated forms of the DNA damage checkpoint kinases CHK1 and CHK2 [34]. It has been shown by chromatin immunoprecipitation and whole-genome scanning approaches that the chromosome ends of senescent cells directly contribute to the DNA damage response and that uncapped telomeres directly associate with many, but not all, DNA damage response proteins [34].

The mitochondrial production of reactive oxygen species (ROS) is one of the causes of replicative senescence [35]. By sorting early senescent (SES) cells from young proliferating fibroblast cultures, it was shown that SES cells have higher ROS levels, dysfunctional mitochondria, shorter telomeres, and telomeric γ-H2A.X foci. The authors propose that mitochondrial ROS is a major determinant of telomere-dependent senescence at the single-cell level that is responsible for cell-to-cell variation in replicative life span [35]. Recent studies confirmed an intimate connection in the normal replicative senescence of human diploid fibroblasts (HDF) between oxidative stress and telomere-dependent effects on cell proliferation. They suggest that mitochondrial dysfunction largely determines the age-related development of the extensive cell-to-cell variation in cell division potential (reviewed in ref. [35]). Replicative senescence in HDF is known to be caused ultimately by a DNA damage response that is triggered by uncapped telomeres [34, 36], which in turn result from replication-driven loss of telomere repeats in the absence of telomerase. This process is sometimes interpreted as “programmed aging” at the cell level, although the idea of an underlying program driving replicative senescence is hard to
reconcile with its extensive, intrinsic heterogeneity. Furthermore, telomere shortening rate and cell replicative life spans can be greatly modified by DNA-damaging oxidative stress (Figure 4) [33] via a telomere-specific repair deficiency, which causes stress-dependent accumulation of single-stranded breaks [37] and accelerates telomere shortening during DNA replication [38]. The frequency of single-stranded regions is significantly higher in telomeres than in minisatellites or in the bulk of the genome under all conditions tested [37]. Those regions induced in minisatellites or in the overall genome by a bolus dose of hydrogen peroxide are completely repaired within 24 h. On the contrary, 50±12% of H₂O₂-induced single-stranded regions remain unrepaired for at least 19 days in telomeres of human fibroblasts, leading to a significant increase of the telomeric steady-state level of these lesions [37]. This preferential accumulation might significantly contribute to telomere shortening [37]. The data suggest that metabolic time-dependent single-stranded degradation is a major cause of telomere shortening. They support the idea that telomere shortening plays an important role in triggering cellular senescence [38].

This has led to the suggestion that telomere reduction is not strictly programmed, with TL acting as a mere cell-division counting device, but instead that telomeres act as sentinels for cumulative oxidative and/or environmental stress triggering division arrest when the damage burden (detected through TL) becomes too great [33].
Telomeres cap and are renowned to protect the ends of chromosomes from degradation and illegitimate recombination. The termini of a linear template cannot, however, be completely replicated by conventional DNA-dependent DNA polymerases; thus, in the absence of a mechanism to counter this effect, telomeres of eukaryotic cells shorten every round of DNA replication. In humans and possibly other higher eukaryotes, telomere shortening may have been adopted to limit the life span of somatic cells [39]. Telomere maintenance is thought to play a role in signaling cellular senescence; however, a link with organismal aging processes has not been established [40]. Normal human somatic cells have a finite life span and undergo replicative senescence after a limited number of cell divisions. Life span appears to be governed by cell division, not time [39]. Erosion of telomeric DNA has emerged as a key factor in senescence, which is antagonized during cell immortalization (Figure 5) and transformation [42].

Telomeric DNA in the skin cells of 21 human subjects aged between 0 and 92 years was quantified by determining the length of the telomeric smear and the relative amount of TTAGGG repeat sequences. Both TL and quantity of telomeric repeat sequences were found to decrease significantly with age. Telomere loss has been postulated to be a cause of cell senescence [43]. The rates of telomere attrition vary markedly at different ages. Telomeric repeats are lost rapidly (at a rate of >1 kb/year) from the peripheral blood leukocytes of young children, followed by an apparent plateau between age 4 and young adulthood, and by gradual attrition later in life. These data suggest that the loss of telomeric repeats in hematopoietic cells is a dynamic process that is differentially regulated in young children and adults. The results have implications for current models of how telomeric sequences are lost in normal somatic cells and suggest that peripheral blood leukocytes are an excellent tissue to investigate how this process is controlled [44].
Introduction of hTERT gene. 1608 – original adult skin fibroblast strain. Fibroblasts stop proliferation after they reach TL at 68 PDs. 1608tel7, 1608tel2, and 1608tel1.2 – different clones. (B) High-density monolayer of telomerized cells. (C) Increasing TL after telomerization (Southern blot). 1, original cells; 2–7, different clones of telomerized cells; left, DNA size markers (kb). (D) Normal karyotype of telomerized cells. (E) Increasing saturated density of proliferation after telomerization. Old cells [strain 1608 57PDL (PD level)] have very low density of monolayer. Young cells (1608 21PDL) form monolayer of increased density, and telomerized cells (1608telmix and 1608telmixclone) form very high density monolayer (also see B) [10]. Telomere shortening occurs concomitant with organismal aging (Figure 6), and it is accelerated in the context of human diseases associated with mutations in telomerase, such as some cases of dyskeratosis congenita, idiopathic pulmonary fibrosis, and aplastic anemia [41].

Multiple interacting components of the telomere, together with telomerase (and sometimes recombination), determine whether a telomere will be functional, allowing cell proliferation [36]. The various components reinforce each other, providing for a robust and resilient system of protection and replenishment of telomeres. A characteristic of a telomere is that its structural features elicit responses that allow it to be dynamically recapped. Eliciting a DNA damage response through uncapping of a telomere appears to be one way in which telomerase action at that telomere is stimulated. Thus, as long as a timely and appropriate recapping of the telomere is possible, regulated uncapping of a telomere appears to be not only normal, but even required for optimal telomere maintenance. TL and the presence of telomerase provide an example of a pair of interacting components that determine telomere capping function. Telomerase is dispensable in cells with sufficiently long telomeres; however, in cells with short telomeres lacking telomerase, cells lose the ability to proliferate, and in some cell types, telomere fusions are increased. However, expressing telomerase can make even very short telomeres functional [36].

Telomere shortening occurs concomitant with organismal aging (Figure 6), and it is accelerated in the context of human diseases associated with mutations in telomerase, such as some cases of dyskeratosis congenita, idiopathic pulmonary fibrosis, and aplastic anemia [41]. People with these diseases, as well as Terc-deficient mice, show decreased life span coincidental with a premature loss of tissue renewal, which suggests that telomerase is rate limiting for tissue homeostasis and organismal survival. These findings have gained special importance, as they suggest that telomerase activity and TL can directly affect the ability of stem cells to regenerate tissues. Stem cell dysfunction provoked by telomere shortening has been proposed as one of the mechanisms responsible for
The authors of this article studied the telomere structures that are part of one’s chromosomes. Individuals are born with telomeres of certain length, and in many cells, telomeres shorten as the cells divide and age. TL is therefore considered a marker of biological aging [7–10, 43, 44]. Advocates of human life extension promote the idea of lengthening the telomeres in certain cells through temporary activation of telomerase (by drugs) or possibly permanently by gene therapy. They reason that this would extend human life. It has been hypothesized that there is a tradeoff between cancerous tumor suppression and tissue repair capacity, in that lengthening telomeres might slow aging and in exchange increase vulnerability to cancer [45]. That concept, coupled with recent findings in oncology and gerontology, provides the foundation for an integrative theory of vertebrate senescence that reconciles aspects of the “accumulated damage”, “metabolic rate”, and “oxidative stress” models. Changing TL is usually associated with changing speed of senescence. This telomere shortening, however, might be a consequence of, and not a reason for, aging.

Organismal aging in both humans and mice [41]. Telomeres shorten at each cell division, in part because the normal DNA replication process does not fully copy the chromosome end [26, 40]. In addition to the end replication problem [26, 41], there is good evidence that telomere erosion is accelerated by other factors, particularly oxidative stress [33, 46]. TL is maintained by a balance between processes that lengthen and those that shorten telomeres. Evidence from various organisms suggests that several factors influence TL regulation, such as telomere binding proteins, telomere capping proteins, telomerase, and DNA replication enzymes. Understanding how these factors interact to coordinate the regulation of TL will allow a more complete understanding of telomere function in the cell and of targeted therapeutics on telomeres and telomerase biology [47]. In human cells, TL is not maintained and telomerase is not active in some tissues. In tumors, however, telomerase is active and may be required for the growth of cancer cells. Telomere shortening in cells with low intrinsic telomerase activity such as fibroblasts is governed by various mechanisms including the so-called end-replication problem, end processing, and oxidative DNA damage [46].

To assess the impact of oxidative stress on telomere shortening rates, a group of authors compared telomere shortening rates measured in fibroblasts from two different donor species (human and sheep) under both prooxidative and antioxidative culture regimens [46]. Over an almost 50-fold change in peroxide indicator dye fluorescence intensity, it has been found a continuous, exponential correlation between cellular oxidative stress levels and telomere shortening rates, which was independent of donor species and cell strain. This correlation suggests that stress-mediated telomere DNA damage is an important determinant of telomere shortening. In vitro studies have shown that, once a critical TL is reached, cells stop dividing and enter a state of replicative senescence, which may be followed by apoptosis [26, 41]. It has been proposed that the finite doubling capacity of normal mammalian cells is due to a loss of telomeric DNA and eventual deletion of essential sequences. In yeast, the est1 mutation causes gradual loss of telomeric DNA and eventual cell death mimicking senescence in higher eukaryotic cells. The amount and length of telomeric DNA in human fibroblasts decreases as a function of serial passage during aging in vitro and possibly in vivo.

It is not known whether this loss of DNA has a causal role in senescence [25].

Analysis of human fibroblast cells from 31 donors (aged 0–93 years) indicated relatively weak correlations between proliferative ability and donor age (m=-0.2 doubling/year; r=-0.42; p=0.02) and between telomeric DNA and donor age (m=-15 bp/year; r=-0.43; p=0.02) [48]. However, there was a striking correlation, valid over the entire age range of the donors, between replicative capacity and initial TL (m=10 doublings/kbp; r=0.76; p=0.004), indicating that cell strains with shorter telomeres
underwent significantly fewer doublings than those with longer telomeres. These observations suggest that TL is a biomarker of somatic cell aging in humans and are consistent with a causal role for telomere loss in this process (Figure 6) [48]. It was also found that fibroblasts from Hutchinson-Gilford progeria donors had short telomeres, consistent with their reduced division potential in vitro. In contrast, telomeres from sperm DNA did not decrease with age of the donor, suggesting that a mechanism for maintaining TL, such as telomerase expression, may be active in germline tissue [48].

Maternal protein undernutrition can influence the growth and longevity. A group of authors has tested the hypothesis that these differences in longevity were associated with changes in the rate of telomere shortening [49]. The authors found age-related shortening of telomeres in the liver and kidney but not in the brain of tested laboratory animals (male rats). Growth retardation in postnatal life was associated with significantly longer kidney telomeres and an increased longevity. Conversely, growth retardation during the fetal life followed by postnatal catchup growth was associated with a shorter life span and shorter kidney telomeres. These findings may provide a mechanistic basis for epidemiological studies linking early growth retardation to adult degenerative diseases [49]. Another group of authors measured TL in erythrocytes from five bird species with markedly different life spans [50]. Species with shorter life spans lost more telomeric repeats with age than species with longer life spans. A similar correlation is seen in mammals. Furthermore, telomeres did not shorten with age in Leach’s storm-petrels, an extremely long-lived bird, but actually lengthened. This novel finding suggests that regulation of TL is associated not only with cellular replicative life span but also with organismal life span and that very long-lived organisms have escaped entirely any telomeric constraint on cellular replicative life span [50].

Age-dependent telomere shortening and accompanying genetic instability were associated with shortened life span as well as a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation. In addition, it was found an increased incidence of spontaneous malignancies. These findings demonstrate a critical role for TL in the overall fitness, reserve, and well-being of the aging organism [40, 51]. A group of authors aimed to assess an association between TL and mortality in 143 normal unrelated individuals over the age of 60 years. Those with shorter telomeres in blood DNA had poorer survival, attributable in part to a 3.18-fold higher mortality rate from heart disease (95% CI, 1.36–7.45; p=0.0079) and an 8.54-fold higher mortality rate from infectious disease (95% CI, 1.52–47.9; p=0.015) [52]. These results lend support to the hypothesis that telomere shortening in human beings contributes to mortality in many age-related diseases. Shorter telomeres are associated with an increased likelihood of mortality, including death from heart disease. Another group of authors examined the association between TL and heart disease (present in 33%) in a well-characterized, narrow age cohort of older people (n=190, all born in 1921) and tested for any concomitant effects of medication use. Mean TL was significantly shorter in participants who reported heart disease (p=0.001) [53]. Participants with ischemic changes on electrocardiography (ECG) had shorter TL (6.67 vs. 7.65 kb; p=0.021) after adjusting for other ECG abnormalities. This finding adds to the growing body of evidence for an association between telomere shortening and ischemic heart disease (reviewed in ref. [53]). Telomere shortening in peripheral blood leukocytes is a promising index of ischemic heart disease risk in older people (for further information, see “Clinical disorders associated with age-dependent telomere loss. Telomerase-based strategies”). The results in the nematode Caenorhabditis elegans suggest that signaling may be initiated in postmitotic somatic cells by TL to regulate organismal life span [54]. There is an evidence linking TL and survival [55].

Studies in a range of organisms have shown that telomeres shorten with age in various somatic tissues [47–52, 54–56], and individuals with relatively long telomeres have a greater life expectancy than those with short telomeres [40, 52, 54–58]. However, such an effect could be a consequence of either an initially long TL or a slow rate of telomere erosion. Although initial TL is partly genetically determined, subsequent accelerated telomere shortening has been linked to elevated levels of oxidative stress. Recent studies show that short TL alone is insufficient to induce cellular senescence; advanced attrition of these repetitive DNA sequences does, however, reflect aging processes. Furthermore, telomeres vary widely in length between individuals of the same age, suggesting that individuals differ in their exposure or response to telomere-shortening stress factors [57].

Elucidation of the regulatory pathways involved in the repression of telomerase activity during development may lead to the ability to manipulate telomerase levels and explore the consequences both for cellular aging and for the survival of cancer cells [59]. Telomerase activation has potential medical applications beyond extending human life span. In this review, we recapitulate the available data, propose a synthetic view of TL control mechanisms in humans, and suggest new therapeutic approaches.
**TL and cellular aging: Olovnikov’s prediction**

In most eukaryotic organisms, telomere shortening can be countered by the de novo addition of telomeric repeats by the enzyme telomerase. Cells that are “immortal”, such as the human germline or tumor cell lines, established mouse cells, yeast, and ciliates, all maintain a stable TL through the action of telomerase. Abolition of telomerase activity in such cells nevertheless results in telomere shortening, a process that eventually destabilizes the ends of chromosomes, leading to genomic instability and cell growth arrest or death. Therefore, loss of terminal DNA sequences may limit cell life span by two mechanisms: by acting as a mitotic clock and by denuding chromosomes of protective telomeric DNA necessary for cell viability [39].

In 1973, Olovnikov proposed that cells lose a small amount of DNA following each round of replication due to the inability of DNA polymerase to fully replicate chromosome ends (telomeres) and that eventually a critical deletion causes cell death [28]. More recently published observations showing that telomeres of human somatic cells act as a mitotic clock, shortening with age both in vitro and in vivo in a replication-dependent manner, support this theory’s premise (Figure 3A–C). In addition, since telomeres stabilize chromosome ends against recombination, their loss could explain the increased frequency of dicentric chromosomes observed in late-passage (senescent) fibroblasts and provide a checkpoint for regulated cell cycle exit. Sperm telomeres are longer than somatic telomeres and are maintained with age, suggesting that germline cells may express telomerase, the ribonucleoprotein enzyme known to maintain TL in immortal unicellular eukaryotes. As predicted, telomerase activity has been found in immortal, transformed human cells and tumor cell lines but not in normal somatic cells. Telomerase activation may be a late, obligate event in immortalization since many transformed cells and tumor tissues have critically short telomeres. Thus, TL and telomerase activity appear to be markers of the replicative history and proliferative potential of cells [60].

Recent progress in determining the roles of various genetic influences in controlling the rate of cellular aging has made this an exciting time in aging research. Aging research within the past years has implicated a variety of mechanisms ranging from the control of gene expression, stress resistance, and DNA metabolism to the overall “rate of living”. The regulation of TL and its role in senescence and cellular immortalization has been found to be more complex than initially expected [61]. Reactivation of telomerase in cultured human cells extends their replicative life span beyond the Hayflick limit. How telomere shortening triggers cell senescence and whether it contributes to aging in vivo are under investigation [62].

**Manipulation with telomeres: elucidation of the regulatory factors involved in the repression or suppression of telomerase activity and the program of cellular senescence**

Telomere dynamics and changes in telomerase activity are consistent elements of cellular alterations associated with changes in proliferative state. In particular, the highly specific correlations and early causal relationships between telomere loss in the absence of telomerase activity and replicative senescence or crisis, on the one hand, and telomerase reactivation and cell immortality, on the other, point to a new and important paradigm in the complementary fields of aging and cancer. Although the signaling pathways between telomeres and transcriptional and cell cycle machinery remain undefined, recently described homologies between telomeric proteins and lipid/protein kinase activities important in chromosome stability provide evidence for the existence of pathways transducing signals originating in chromosome structure to cell cycle regulatory processes. Similarities between cell cycle arrest at senescence and the response of mortal cells to DNA/oxidative damage suggest an overlap in the signal transduction mechanisms culminating in irreversible and stable cell cycle arrest [63].

Cell division occurs during life in many tissues, either as part of normal tissue function or in response to tissue damage. The accumulation of cells at the end of their replicative life span in the elderly might contribute to aged tissue either because of a reduced ability to undergo proliferation or because of the known altered gene expression patterns of senescent cells. This has been illustrated experimentally using a transgenic telomerase-negative mouse, which shows some premature aging phenotypes. The mechanism whereby cells count divisions uses the gradual erosion of the ends of chromosomes (telomeres) with cell division caused by the repression of the telomere maintenance enzyme telomerase in most human cells [64]. The aging process has multiple causes. However, there is now substantial evidence consistent with the hypothesis that (a) all normal mammalian somatic cells have a
finite capacity to replicate and (b) gradual cell turnover throughout the life span of a mammal eventually exhausts this finite capacity. This results in a gradual accumulation of senescent (irreversibly postmitotic) cells with increasing age. These cells display a radically different phenotype to their growing counterparts, which has the potential to compromise tissue function. Perhaps the best evidence for this is seen in Werner’s syndrome, a rare genetic disease, in which patients display most of the features of accelerated aging, together with a profoundly compromised replicative life span in certain tissue lineages. Several classes of human cells are now known to count divisions by monitoring the progressive attrition of chromosomal ends (telomeres), leading to the activation of a p53-p21(WAF1)-dependent G1 checkpoint. Ectopic expression of telomerase has been shown to prevent senescence in several cell types and offers the potential for interventions in the aging process based on tissue engineering, gene therapy, or homografts. However, this telomere-driven senescence mechanism seems to be absent from rodents, which use telomere-independent means (perhaps based on p14arf) to count divisions. Similar senescence pathways are now being reported in humans, and this, coupled with the demonstration of tissue-specific telomeric loss rates, has the potential to render strategies based on the use of telomerase dependent on the characteristics of the target tissue [65].

In primary human cells, telomeres shorten with passage in culture, and progressive telomere shortening ultimately limits the replicative capacity of cultured cells [25, 39, 48, 66, 67]. The published findings strongly suggest that the proliferative potential of most, if not all, hematopoietic stem cells is limited and decreases with age, a concept that has widespread implications for models of normal and abnormal hematopoiesis as well as gene therapy [68]. To clarify the involvement of telomerase in the immortalization of keratinocytes, the catalytic subunit of telomerase (hTERT) expression was restored in normal human esophageal epithelial cells (EPC2). EPC2-hTERT cells overcame senescence and were immortalized without p16INK4a genetic or epigenetic alterations. p16INK4a was expressed at moderate levels and remained functional as evidenced by induction with ultraviolet (UV) treatment and binding to cyclin-dependent kinase 4 and 6. There were no mutations in the p53 gene, and p53 was functionally intact. Importantly, senescence could be activated in the immortalized EPC2-hTERT cells by overexpression of oncogenic H-ras or p16INK4a. Furthermore, the EPC2-hTERT cells yielded basal cell hyperplasia in an innovative organotypic culture system in contrast to a normal epithelium from parental cells. These comprehensive results indicate that the expression of telomerase induces immortalization of normal human esophageal keratinocytes without inactivation of p16INK4a/pRb pathway or abrogation of the p53 pathway [43].

It has been repeatedly suggested that telomere-associated cellular senescence may contribute to certain age-related disorders, including an increase in cancer incidence, wrinkling and diminished skin elasticity, atherosclerosis, osteoporosis, weight loss, age-related cata
tact, and others [7, 8, 69]. Afterwards, it was documented that although aged organ systems function adequately to maintain baseline health, short telomeres could be linked more directly to a fundamental feature of aging: a reduced capacity to respond to acute and chronic illness (see “Clinical disorders associated with age-dependent telomere loss: telomerase-based biological strategies”).

One attractive model is that some type of biological clock controls the rate of aging, including the time at which age-related processes such as human puberty and menopause take place. Such a clock would presumably run at vastly different rates in different organisms, since, for example, mice live 2 years, finches live 10 years, and bats live 30 years or more [12, 70]. Less clear, however, is the relevance of progressive telomere shortening as a potential factor in organismal aging [40, 62]. Many processes have been proposed to influence life span, including a failure to replicate telomeres, to withstand oxidative damage, or to combat infectious agents effectively [12, 70]. However, which, if any, of these processes actually determines the rate of aging is unknown. In many instances, an effective way to dissect a regulatory process has been to identify mutations that alter it. If there are proteins that determine the life span of an animal, then it should be possible to mutate the genes encoding these proteins in such a way that the rate of aging is changed. Many diverse genes, such as those involved in gene silencing, DNA repair, genomic stability, and growth factor signaling, have emerged as strong determinants of life span in a variety of species [40, 59, 61, 70, 71].

The SAM strain of mice is actually a group of related inbred strains consisting of series of accelerated senes
cence-prone, short-lived (SAMP) and accelerated senes
cence-resistant, longer-lived (SAMR) strains. Compared with the SAMR strains, the SAMP strains of mice show a more accelerated senescence process, shorter life span, and an earlier onset and more rapid progress of age

associated pathological phenotypes similar to several geriatric disorders observed in humans, including senile osteoporosis, degenerative joint disease, age-related deficits in learning and memory, olfactory bulb and forebrain atrophy, presbycusis and retinal atrophy, senile
amyloidosis, immunosenescence, senile lungs, and diffuse medial thickening of the aorta. The higher oxidative stress observed in the SAMP strains of mice are partly caused by mitochondrial dysfunction and may be one cause of the senescence acceleration and age-dependent alterations in cell structure and function, including neuronal cell degeneration. This senescence acceleration was also observed during senescence/crisis in cultures of isolated fibroblast-like cells from SAMP strains of mice and was associated with a hyperoxidative status [59, 72, 73]. Accelerated changes in the DNA ploidy associated with in vitro aging were examined in fibroblast-like cells isolated from the dorsal dermis of newborn SAMP11 mice and were compared to changes observed in cell lines from SAMR1 mice [73]. Although these changes were observed in the cell lines from both strains of mice, the changes occurred more rapidly and at earlier PDs in the cell lines from the SAMP11 mice. These results suggest that the cell lines from SAMP11 mice might have higher susceptibility to factors that cause polyploidization, including oxidative stress [73].

Whereas somatic cells do not express the enzyme, telomerase, which adds repeated telomere sequences to chromosome ends, telomerase activity is detected in immortalized and tumor cells in vitro and in primary tumor tissues. This represents an important difference between normal cells and cancer cells, suggesting that telomere shortening causes cellular senescence [74]. Immortal and cancer cells compensate for telomeric loss by expressing the enzyme telomerase, an RNA-dependent DNA polymerase that maintains TL. Telomerase activity has been detected in almost 90% of all human cancers. Telomerase activity is generally absent in normal somatic tissues but is detected in adult testes and activated lymphocytes, and lower levels are expressed in proliferative cells of renewal tissues. Telomerase activity is down-regulated in cells that exit the cell cycle via either terminal differentiation or (reversible) quiescence. Inhibition of telomerase activity in tumor cells may provide an effective way to treat cancer by potentially reducing the recurrence of tumors due to occult micrometastases. An understanding of the pathways involved in telomerase regulation will be important for determining the most practical means of inhibiting its activity [75].

Hybrids between immortal cells and normal cells senesce, indicating that immortal cells have lost, mutated, or inactivated genes that are required for the program of senescence in normal cells. Genes involved in the senescence program have been mapped to over 10 different genetic loci using microcell fusion to introduce human chromosomes and restore the senescence program. Multiple pathways of cellular senescence have also been demonstrated by chromosome transfer, indicating that the functions of the mapped senescence genes are probably different. One possibility is that one or more of these senescence genes may suppress telomerase activity in immortal cells, resulting in telomere shortening and cellular senescence [74]. To test this hypothesis, telomerase activity and the length of terminal restriction fragments (TRFs) have been examined in microcell hybrids. The loss of indefinite growth potential was either with or without the loss of telomerase activity and shortening of telomeres in the microcell hybrids containing the introduced chromosome. The findings suggest that telomerase regulation is one of multiple pathways to cellular senescence [76].

Some time ago, a group of authors have reported the inhibition of human telomerase activity by peptide nucleic acids (PNAs). PNAs recognize the RNA component of human telomerase (hTR) and inhibit activity of the enzyme with IC₅₀ values in the picomolar to nanomolar range. Inhibition depends on targeting exact functional boundaries of the hTR template and is 10- to 50-fold more efficient than inhibition by analogous phosphorothioate (PS) oligomers [77]. In contrast to high selectivity of inhibition by PNAs, PS oligomers inhibit telomerase in a non-sequence-selective fashion. These results demonstrate that PNAs can control the enzymatic activity of ribonucleoproteins and possess important advantages relative to PS oligomers in both the affinity and the specificity of their recognition. These observations should facilitate the development of effective inhibitors of telomerase activity and affinity probes of telomerase structure [77].

Telomerase is kept in control by the protein TRF1, which keeps the telomeres operating correctly. However, another protein, Fbx4, can bind to TRF1 and degrade it, causing the telomeres to lengthen. TRF1 is a critical regulator of TL. As such, TRF1 levels are regulated by ubiquitin-dependent proteolysis via an SCF E3 ligase, where Fbx4 contributes to substrate specification. The crystal structure of the Fbx4-TRF1 complex at 2.4 Å resolution has been discovered [78]. Fbx4 contains an unusual substrate-binding domain that adopts a small GTPase fold. Strikingly, this atypical GTPase domain of Fbx4 binds to a globular domain of TRF1 through an intermolecular β sheet instead of recognizing short peptides/degrons as often seen in other F-box protein-substrate complexes. Importantly, mutations in this interface abrogate Fbx4-dependent TRF1 binding and ubiquitination [78]. Now, researchers have discovered that a third protein, TIN2, can step in and override Fbx4 by binding to TRF1 first and preventing Fbx4 from attaching to it [78]. The data demonstrate that recognition of TRF1 by SCF(Fbx4) is regulated
by this telomere protein, TIN2. The results reveal an atypical small GTPase domain within Fbx4 as a substrate-binding motif for SCF(Fbx4) and uncover a mechanism for selective ubiquitination and degradation of TRF1 in telomere homeostasis control. This finding paves the way for developing a drug that acts like TIN2, keeping everything in check and stopping the first “domino” from falling. The researchers are now looking at peptides that mimic TIN2’s binding to TRF1 in order to block Fbx4. The work is still in preliminary stages and no new therapies are being tested in patients [78].

Hybrids between immortal cells that express telomerase and normal cells that lack telomerase have a limited life span. It has been demonstrated that telomerase is repressed in such hybrids [79]. Treatment of immortal human cell lines with certain oligonucleotides resulted in telomere elongation. The authors of the study [79] took advantage of this observation to test the hypothesis that elongation of telomeres would extend the life span of cells in culture. An immortal human cell line was treated with an oligonucleotide to lengthen its telomeres and then was fused with mortal cells. The life span of these hybrid cells was longer than that of the hybrids in which telomeres had not been elongated. These observations provide the direct evidence supporting the hypothesis that TL determines proliferative capacity of human cells [79]. Elucidation of the regulatory pathways involved in the repression of telomerase activity during development may lead to the ability to manipulate telomerase levels and explore the consequences both for cellular aging and for the survival of cancer cells [71]. In contrast, the tight relationship between replicative senescence and telomere shortening in cultured human cells has led to the view that TL regulation may provide a molecular explanation for diminished reserve and cellular senescence in aged tissues [8, 29–31, 43–45, 48, 62, 63, 68, 80–82]. Telomere erosion ultimately triggers replicative senescence in many cell types; this can be prevented experimentally by forcibly expressing telomerase. This extends the life span of normal human cells. Telomere-driven senescence did not evolve to cause aging but is instead a byproduct of a system devised to provide a tumor suppression function, a concept that fits well with evolutionary arguments regarding tradeoffs between somatic maintenance and reproduction [64].

The telomere hypothesis of aging and immortalization postulates that sufficient telomere loss on one or more chromosomes in normal somatic cells triggers cell senescence, whereas reactivation of the telomerase enzyme is necessary for cell immortalization. Measurements of TL and telomerase activity in cancer and during normal and accelerated human aging in skin, blood, hemopoietic, skeletal muscle, vascular, and central nervous system (CNS) tissues support this model. Tissue culture studies of cell aging and transformation have added to our understanding of telomere dynamics in these processes. Evolution of telomerase repression and mortality in somatic cells of long-lived organisms is consistent with antagonistic pleiotropy considerations in which cell senescence is a tumor suppressor mechanism: stringent repression of telomerase has a beneficial early effect in reducing the probability of cancer but a deleterious, unselected late effect in its contributions to age-related disease [81]. Antitelomerase strategy for cancer therapy is attractive but limited by the short decrease of the TL at each cell division [83]. The feasibility of targeting telomeres/telomerase as a strategy for antiproliferative therapeutics has been shown in studies in yeast, in which mutations in specific telomere associated genes result in delayed cell death. Similarly, antisense oligonucleotide inhibition of telomerase activity in human tumor cells (HeLa) results in delayed cell death (reviewed in ref. [63]). The mechanism of cell death and possible escape from this fate require further study. In human cells, however, it would seem reasonable to predict that, in these circumstances, apoptosis is induced in the vast majority of cells either directly in response to a DNA damage signal arising from critically shortened telomeres or as a secondary consequence of genetic instability [63].

Clinical disorders associated with age-dependent telomere loss: telomerase-based biological strategies

Can telomere dynamics, defined by TL and attrition rate, provide information about the biology of human aging above and beyond that provided by chronological age? Accruing data suggest that it can. White blood cells (WBCs) have been used as the primary model in attempts to decipher links between aging, aging-related disorders, and telomere dynamics in humans [84]. The WBC model may be appropriate in clinical settings, provided that we fully appreciate its drawbacks and limitations. On the basis of WBC telomere data, it is evident that age-adjusted TL is highly variable, highly heritable, longer in women than men, and shorter in people who harbor a host of age-related disorders, whose common denominators may prove to be increased oxidative stress and inflammation. It appears that shorter age-adjusted WBC TL augurs a
greater risk of morbidity and premature mortality in the elderly [84].

Telomere shortening is a marker of aging; therefore, TL might be related to disease progression and survival [85]. Because telomeres are eroded during mitosis, TL indicates the replicative history of human somatic cells [86]. Critically short telomeres may trigger replicative senescence, albeit other processes, including the capped/uncapped telomeric status and telomerase activity, are major determinants in this phenomenon. Thus, TL largely registers the replicative history, the cumulative oxidative burden, and, in part, the proliferative potential of somatic cells in culture [87]. The experiments show that the rate of telomere shortening in vitro is modulated by oxidative stress as well as by differences in antioxidative defense capacity between cell strains [88].

In humans, telomeres are short, and telomerase activity is low or undetectable in many somatic tissues but present in germ cells, activated leukocytes, and stem cells from a variety of organs [59, 89, 90]. However, the degree to which telomerase maintains telomeres in these tissues during aging has not yet been fully explored. Repression of telomerase activity in human somatic cells, which leads to telomere shortening and replicative senescence, may have evolved as a protective mechanism against immortalization, unfettered clonal evolution, and cancer [90]. Evidence from various organisms suggests that several factors influence TL regulation, such as telomere binding proteins, telomere capping proteins, telomerase, and DNA replication enzymes. Understanding how these factors interact to coordinate the regulation of TL will allow a more complete understanding of telomere function in the cell [47].

Epidemiological studies have confirmed that short telomeres in humans are a risk factor for diseases including, among others, atherosclerosis, diabetes, Alzheimer’s disease, and cancer. We specify that age-dependent telomere loss may contribute to a reduction in viable cells, altered differentiation functions, and impaired regenerative/proliferative responses, particularly in the settings of stress such as those seen in chronic infections, cirrhosis, chronic skin ulcerations, and hypertensive vascular injury, among others [91–93]. The previously published results show that endothelial cells lose telomeres in vitro as a function of replicative age and that telomere loss, in vivo, is generally greater in those tissues involved in or susceptible to atherogenesis [85]. These data document that TL can be employed to monitor the replicative history of tissues implicated in atherosclerosis and that replicative senescence of vascular cells may play a critical role in atherogenesis [91]. The TL measured in patients with chronic hepatitis or liver cirrhosis revealed a significant telomere shortening in the liver with chronic liver disease compared to that in the normal liver. The TL tended to decrease with the progression of chronic liver disease [92]. The aim of the separate study was to elucidate the role of the glutathione-dependent antioxidant system on the replicative capacity and telomere dynamics of cultured endothelial cells. The findings demonstrate a key role for glutathione-dependent redox homeostasis in the preservation of telomere function in endothelial cells and suggest that loss of telomere integrity is a major trigger for the onset of premature senescence under mild chronic oxidative stress [94].

The strongest evidence that cellular aging, as reflected by shorter telomeres, might be associated with organismal aging has until now been derived from cross-sectional studies. Shorter TL in leukocytes has been associated cross-sectionally with CVD and its risk factors, including pulse pressure and vascular aging [1, 86, 95–97], obesity [87, 98, 99], vascular dementia [88, 100], diabetes [98, 101–104], coronary artery disease (CAD) [1, 105, 106], myocardial infarction (MI) [107] although not in all studies [108], and cellular turnover and exposure to oxidative and inflammatory damage in chronic obstructive pulmonary disease (COPD) [109]. Leukocyte TL correlates with a subset of measures of cognitive performance, suggesting that it might be a biomarker of cognitive aging in women before the onset of dementia [110]. TL has been shown to predict CVD events (MI and stroke) in men younger than 73 years old [111]. These results support the hypotheses that telomere attrition may be related to diseases of aging through mechanisms involving oxidative stress, inflammation, and progression to CVD [111]. The longer TL in women suggests that, for a given chronological age, biological aging of men is more advanced than that of women [96]. Telomeres are remarkably shorter in patients with aging associated diseases, including CAD and chronic heart failure. In addition, numerous conventional cardiovascular risk factors are associated with shorter TL [106]. If telomeres can be proven to be not only associated but also causally involved in the pathogenesis of CVD, it might provide exciting new avenues for the development of future preventive and therapeutic strategies [112, 113].

Obesity and smoking are important risk factors for many age-related diseases. Both are states of heightened oxidative stress, which increases the rate of telomere erosion per replication, and inflammation, which enhances WBC turnover [9, 10, 98, 99]. Obese adults have shorter telomeres than their normal-weight counterparts, while this phenomenon is not present in childhood [98]. Together,
these processes might accelerate telomere erosion with age [98, 99]. Monocyte telomere shortening in type 2 diabetes could be due to increased oxidative DNA damage to monocyte precursors during cell division [102]. These data suggest that monocytes adhering to vascular endothelium and entering the vessel wall in type 2 diabetes are from a population with shorter telomeres and at increased risk of replicative senescence within vascular plaque [102]. Young adult mice that are deficient for the Terc subunit of telomerase exhibit impaired glucose tolerance. This is caused by impaired glucose-stimulated insulin secretion (GSIS) from pancreatic islets, while body fat content, energy expenditure, and insulin sensitivity were found to be unaltered. The impaired secretion capacity for insulin is due to reduced islet size, which is linked to an impaired replication capacity of insulin-producing β-cells in Terc-deficient mice. It has been established that telomerase deficiency and hence short telomeres impaire replicative capacity of pancreatic β-cells to cause impaired insulin secretion and glucose intolerance, mechanistically defining diabetes mellitus as an aging-associated disorder [104].

Dietary restriction (DR) in mice improved telomere maintenance in the liver and intestine and reduced cumulative oxidative stress markers in the same tissue compartments without increased telomerase activity [114]. The authors propose [114] that reduction of cell senescence might be a primary effect of DR, which may explain improved mitochondrial function and reduced ROS production.

Collectively, the former observations suggest that hypertension, increased insulin resistance, and oxidative stress are associated with shorter leukocyte TL and that shorter leukocyte TL in hypertensives is largely due to insulin resistance [115].

The recently published results provided only partially a support to the hypothesis that telomere shortening in human beings contributes to mortality in many age-related diseases [52]. Telomeres prevent the loss of coding genetic material during chromosomal replication. Previous research suggests that shorter TL may be associated with lower survival. Because genetic factors are important for individual differences in both TL and mortality, this association could reflect genetic or environmental pleiotropy rather than a direct biological effect of telomeres. Bakaysa et al. [116] have demonstrated through within-pair analyses of Swedish twins that TL at advanced age is a biomarker that predicts survival beyond the impact of early familial environment and genetic factors in common with TL and mortality. Cawthon et al. found that TL predicted earlier mortality, particularly from CVD and infectious disease, in a sample of 143 healthy men and women 60 years and older [52]. This suggests that poor telomere maintenance may serve as a prognostic biomarker of risk of early mortality. Since then, additional studies have found blood TL predicts mortality, in large twin studies [116, 117], in patients with Alzheimer’s disease and dementia [118], and in stroke patients [119]. It has been initially proposed that TL is a prognostic marker for poststroke cognitive decline, dementia, and death [119]. However, other reports, notably those with very elderly cohorts, have failed to find an association between TL and mortality [120, 121]. Longitudinally, TL was highly unstable in a large fraction of participants (598 participants at baseline). The authors have concluded that blood monocyte TL is not a predictive indicator for age-related morbidity and mortality at ages over 85 years possibly because of a high degree of TL instability in this group [121]. Shortened mean leukocyte (and lymphocyte) TL in a middle-aged male who dies from atherosclerotic coronary heart disease probably is not the cause of his demise but is a lengthy record of underlying processes that have led to his disease [117]. Leukocyte telomere dynamics might help explain the boundaries of the human life span [117]. Telomere dysfunction can provoke chromosomal fusions and apoptotic cell death [122].

We conclude in this section that telomere homeostasis is regulated through mutually reinforcing mechanisms, such as its precise protein composition, TL, and telomerase activity level. The probability of telomere uncapping increases when one or more of these parameters are critically altered and cannot be compensated by the others. For instance, telomerase is dispensable in cells with sufficiently long telomeres, but cells with critically short telomeres that lack telomerase lose their ability to proliferate (replicative senescence). In humans, TL is largely genetically determined but also featured by an age-dependent attrition. TL has therefore been put forward as a marker for biological aging and was also reported to be associated with aging diseases such as CVD. However, it remains unclear whether the biomarker value in a particular disease depends on shorter TL at birth or rather if it is a mere reflection of an accelerated telomere attrition during lifetime or, else, if it is a combination of both [123]. Comparison of the species-specific rates of telomere erosion calculated from the TL in different age categories suggests that telomere erosion rate might be most important [50, 124]. The apparent evolutionary conservatism in the rate of increase in mortality with age suggests that variation in the rate of senescence reflects fundamental changes in organism structure, likely associated with the rate of development, rather than physiological or biochemical processes influenced by a few genes. Understanding
these evolved differences between long-lived and short-lived organisms would seem to be an essential foundation for designing therapeutic interventions with respect to human aging and longevity [125]. While the importance of telomere attrition is supported by cross-sectional evidence associating shorter telomeres with oxidative stress and inflammation, longitudinal and drug evaluation studies are required to accurately assess and target therapeutically telomere attrition, telomerase activity, and their presumed link with accelerated aging. In this section, we have presented different disorders for the biomarker value of TL and discussed the clinical considerations of studying TL in a longitudinal population study, with a special emphasis on treatment of age-related diseases, CVD, obesity, and diabetes problems and mortality.

Telomere shortening, telomerase enhancers, and immune system

One might anticipate that high turnover organs such as the skin, lymphoid, and gastrointestinal tract would be more adversely affected due to an accelerated loss of telomere repeats [125]. The altered differentiation may be critical for compromising the function and integrity of organs such as the skin during aging. Senescent keratinocytes and fibroblasts appear to accumulate with age in human skin. Moreover, senescent cells express genes that have long-range, pleiotropic effects – degradative enzymes, growth factors, and inflammatory cytokines. Thus, relatively few senescent cells might compromise skin function and integrity. Moreover, by altering the tissue microenvironment, senescent cells may also contribute to the rise in cancer that occurs with age [125].

The present study also reports encouraging information on the effect of controlled telomerase activators on the body’s immune system. Infectious diseases lead to telomere shortening in the immune system, as immune cells divide to fight infections. Telomerase activation should prevent this telomere shortening and allow the body’s immune system to fight a chronic infection indefinitely. Telomerase activation is a potential treatment for AIDS [126]. Although improved immunity cannot be considered a “cure” for aging and disease, the beneficial effects promise to enhance health and quality of life of the elderly and of persons infected with HIV-1, a disease characterized by accelerated aging of the immune system. The experiments confirm the utility of telomerase enhancers in antiviral immune function, and they will be developed further for clinical use as a treatment for HIV/AIDS.

The immune status of elderly humans is the composite outcome of genetic background, thymic involution, and, most importantly, a lifelong exposure to a myriad of encounters with foreign pathogens. The immunological history of humans cannot, therefore, be mimicked in laboratory animals, which are subject to minimal exposure to such antigens [126]. Due to the extraordinarily low frequency of T cells specific for any single antigen, immune responses require a massive degree of proliferation in order to adequately control infection. In situations involving chronic antigenic stimulation, such as infection with certain viruses that establish latency, some of the responding CD8 T cells eventually reach their innate proliferative limit by a telomere-based process known as replicative senescence. Cells with characteristics suggestive of replicative senescence accumulate progressively with age, and, in accelerated fashion, during chronic infection with HIV-1. High proportions of these cells are correlated with a variety of deleterious health outcomes, including poor control over infection, reduced responses to vaccines, bone loss, and early mortality. In addition, the presence of large numbers of senescent T cells may affect naive and memory T-cell populations via homeostatic mechanisms that regulate the size and composition of the T-cell pool [126].

Cells of the immune system are unique among normal somatic cells in that they have the capacity to up-regulate the telomere-extending enzyme, telomerase, albeit in a precisely controlled fashion. Kinetic analysis of telomerase activity in long-term T-cell cultures has documented that the high level of telomerase induced in concert with activation reaches a peak at 3–5 days and then declines by 3 weeks. The process is recapitulated during secondary antigenic stimulation, but by the third, and all subsequent stimulations in vitro, CD8 T cells are unable to up-regulate telomerase. Cell division in the absence of telomerase activity results in progressive telomere shortening and, ultimately, the DNA damage/cell cycle arrest that is signaled by critically short telomeres [126]. Gene therapy of HIV-specific CD8 T cells with the telomerase catalytic component (hTERT) results in enhanced proliferative capacity, increased antiviral functions, and a delay in the loss of CD28 expression, with no changes in karyotype or growth kinetics.

Development of methods to reverse and/or prevent replicative senescence is an important facet of aging research due to both its potential use in maintaining function and its possible role in tissue regeneration. Based on the pivotal role of telomere shortening in the replicative senescence program, efforts to manipulate the process have focused attention on the telomere extending enzyme, telomerase. Early studies, using gene
transduction with the catalytic component of human telomerase (hTERT), were performed on a variety of non-immune cell types, including human fibroblasts, epithelial cells, and keratinocytes (Figure 5) [127]. Expression of the catalytic subunit of human telomerase, hTERT, extends human primary fibroblast life span (Figure 5). Such life-span extension has generally been reported to be accompanied by net telomere lengthening, which led to the hypothesis that it is the telomere lengthening that causes the life-span extension [128]. In these experiments, introduction of the hTERT gene led to unlimited proliferation, TL stabilization, normalization of function, and, importantly, no evidence of altered growth or tumorigenesis in immunodeficient mice. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine [127]. These results uncover further hTERT allele-specific phenotypes that uncouple telomerase activity, net telomere lengthening, and life-span extension [128].

Moreover, similar therapeutic approaches may enhance viral immunity and response to vaccines in the elderly. Finally, cancer immunotherapy, which requires prolonged and continuous proliferation and function of tumor-specific CD8 T cells, may also benefit from telomerase activators [126, 129]. The ability of reduce the rate of generation of senescent T cells may result in significantly enhanced immune function and may also correct some of the deleterious effects associated with high proportions of these cells. These proof-of-principle studies have led to screening for pharmacological approaches that might mimic the gene therapy effects in a more clinically suitable formulation [126, 130].

**Imidazole-containing dipeptide-based compounds reduce telomere damage and shortening rate. Advanced oral nutritional support with non-hydrolized forms of carnosine is a therapeutic tool of a critical telomere length maintenance that may prolong life expectancy and increased survival of an organism in health and disease**

Carnosine (β-alanyl-L-histidine) and related compounds are natural constituents of excitable tissues possessing diverse biological activities [131, 132]. The level of carnosine in tissues is controlled by a number of enzymes transforming carnosine into other carnosine related compounds, such as carcinine, N-acetylcarnosine, anserine, or ophidine (by decarboxylation, acetylation, or methylation, respectively) or its cleavage into the amino acids histidine and β-alanine. Hydrolysis is mainly due to tissue carnosinase (EC 3.4.13.3), which is widely distributed among different subjects [133–135], or serum carnosinase (EC 3.4.13.20), which is present in the brain and blood plasma of primates and humans [135–137]. Carnosine has been proven to scavenge ROS as well as α-β unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress [138, 139]. It can oppose glycation [140, 141] and it can chelate divalent metal ions [142]. The important studies have produced clinical and experimental evidence of beneficial effects of N-acetylcarnosine in treating cataracts of the eyes; these and other ophthalmological benefits have been proven [14, 15, 143–151]. Carcinine (β-alanyl histamine) is an imidazole dipeptide first discovered in the crustacean *Carcinus maenas* (reviewed in refs. [132, 135, 152]) and has subsequently been found in the hearts of several mammalian species [153, 154]. It has been demonstrated that carcinine is metabolically related to histamine, histidine, and carnosine (β-alanyl histidine) and could be synthesized from histamine and β-alanine [155]. In addition, previous studies have shown that carcinine contains an imidazole group with flexible ethylene side chain known to be important for histamine H3 receptor-ligand interactions [156–159]. From these findings, it seems that a certain relationship exists between brain histamine and carcinine and that carcinine might be a new histamine H3 receptor antagonist. The results of the recent study provide direct evidence that carcinine, as a novel histamine H3 receptor antagonist, plays an important role in histaminergic neurons activation and might be useful in the treatment of certain diseases, such as epilepsy, and locomotor or cognitive deficit [160]. In two of the reports, carcinine was shown to act as a natural antioxidant [161, 162] and to play a role in regulating stress and shock with a 1000-fold less potent hypotensive effect than histamine [154, 163], suggesting that carcinine might have therapeutic use (reviewed in ref. [164]). Overall, these low molecular mass antioxidant peptidomimetics add significantly to the host defense provided by the enzymes superoxide dismutase, catalase, and glutathione peroxidase and act as pharmacological chaperones in biological systems [132, 135, 161, 162, 165].

A striking antisenescence effect of carnosine was demonstrated by McFarland and Holliday [166–168].
They showed that HDF grown in 20 mM carnosine had an extended life span in both PDs and chronological time. Our own studies of the effects of L-carnosine on human bone marrow stromal cell proliferation demonstrate that L-carnosine increases a proliferative potential of the human bone marrow cells up to three PDs, the phenomenon that can be further exploited in rejuvenation and resuscitation cellular and tissue engineering studies (Figure 7). It was shown that an intake of carnosine in a dose of 50–100 mg/kg of body weight before X-ray irradiation resulted in an increase of the survival of experimental mice. The protective effect of carnosine was manifested, when it was injected either before or after irradiation, but the effect was more pronounced in the case of shortening time between irradiation and injection. An enhancement of colony-forming index of bound cells in spleen was also observed simultaneously with protective action of carnosine [169].

In the recent work, the authors studied the effect of carnosine on a human fetal lung fibroblast strain (HPF), which was either kept in a continuously proliferating or proliferation-inhibited state. The results indicate that carnosine can reduce telomere shortening rate possibly by protecting telomere from damage [170]. Based on our own studies, we have suggested that the reduction in telomere shortening rate and damages in telomeric DNA made an important contribution to the life-extension effect of carnosine and carcinine [9, 10]. Cumulatively, in this article, we propose that patented specific oral formulations of nonhydrolyzed carnosine and carcinine provide a powerful tool for targeted inhibition of cumulative oxidative stress and inflammation associated with telomere shortening in age-related diseases and during smoking behavior (Figure 8, Table 1) [9, 10, 15, 132, 135, 143, 171]. This product concept implements the potential role of the
new nutritional strategy on redox balance in age-related eye diseases and detail how the synergism and interaction of imidazole-containing amino acid-based compounds (nonhydrolyzed L-carnosine, histidine), chaperone agents (L-carnosine, D-pantethine), glutathione-boosting agents (N-acetylcysteine, vitamin E, methionine), and N-acetylcarnosine eye drops plays key roles in the function and maintenance of the redox systems in the aging eye and in the treatment of human cataract disease. A novel patented oral health supplement is presented, which enhances the antcataract activity of eye drops and activates functional visual acuity. The clinical data demonstrate the effectiveness and safety of a combined oral health-care treatment with amino acids possessing chaperone-like activity with N-acetylcarnosine lubricant eye drops (Figure 8). L-carnosine and N-acetylcarnosine protected the chaperone activity of α-crystallin and reduced the increased post-translational modifications of lens proteins. Biological activities of the nonhydrolyzed carnosine in the oral formulation are based on its antioxidant and antiglycating (transglycating) action that, in addition to heavy metal chelation and pH-buffering ability, makes carnosine an essential factor for preventing sight-threatening eye disorders having oxidative stress in their pathogenesis, neurodegeneration, and accumulation of senile features. The findings suggest that synergism is required between carnosine or other imidazole-containing compounds and reduced glutathione in tissues and cells for efficacious protection from protein carbonylation as a biomarker for the ability of the nontoxic compounds to reduce oxidative stress [9, 10, 15, 132, 135, 143, 171].

Potential therapeutic applications of oral forms of nonhydrolyzed carnosine and their specific mechanisms to manage telomere attrition and vascular aging might help elderly patients and smokers to withstand the pathogenetic problems of age-related disorders, metabolic and smoking-related diseases, accompanying with cumulative oxidative stress, accumulation of advanced glycation products, and accelerated biological aging linked with earlier onset of diseases [9, 10, 132, 135, 143, 171].

Conclusions

Telomere maintenance is thought to play a role in signaling cellular senescence; however, a link with organismal aging processes has not been established. In various sections of this review article, we have documented that shortened mean leukocyte (and lymphocyte) TL has been found to be associated with a host of aging-related diseases and lifestyle factors, including CVD, dementia, obesity and insulin resistance, cigarette smoking, psychological stress, and low socioeconomic status, all of which diminish the human life span (age of death from natural causes). Moreover, both life span and TL are longer in women than in men. Leukocyte telomere dynamics might help explain the boundaries of the
Table 1  Can-C™ Plus description.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Daily value</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylcysteine</td>
<td>600 mg</td>
<td></td>
</tr>
<tr>
<td>L-histidine</td>
<td>300 mg</td>
<td></td>
</tr>
<tr>
<td>L-carnosine</td>
<td>210 mg</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150 IU</td>
<td>498%</td>
</tr>
<tr>
<td>D-pantethine</td>
<td>90 mg</td>
<td>894%</td>
</tr>
<tr>
<td>L-methionine</td>
<td>75 mg</td>
<td></td>
</tr>
<tr>
<td>Zinc picolinate</td>
<td>15 mg</td>
<td>99%</td>
</tr>
</tbody>
</table>

*Not established. Other ingredients: Gelatin capsule. Note: Keep out of reach of children. Not for use by pregnant or lactating women. Directions: Take 1 capsule two to three times daily for vision care and other therapeutic purposes and 1 capsule every other day for rejuvenation purposes and prevention of organism aging. Supplement facts: Serving size: 3 capsules Can-C™ Plus has been formulated, approved, and patented by IVP, the inventors of the Can-C™ eye drop technology. Designed to be used as a separate standing therapeutic product with the role of nutritional supplementation in prevention of onset or progression of age-related and ocular disease, it is of interest to health-care professionals and patients. In combined therapeutics with N-acetylcarnosine lubricant eye drops, Can-C™ Plus helps to support the effectiveness and optimizes the action of Can-C™ eye drops in the treatment of sight-threatening eye diseases accompanied with the senescent cellular phenotype. The findings suggest that synergism is required between carnosine or other imidazole-containing compounds and reduced glutathione in tissues and cells for efficacious protection from protein carbonylation as a biomarker for the ability of the nontoxic compounds to reduce oxidative stress. The results show that combining imidazole-containing compounds at near physiological concentrations results in heightened synergistic antioxidant activity [171]. Potential therapeutic applications of oral forms of nonhydrolyzed carnosine and their specific mechanisms to manage telomere attrition and vascular aging might help elderly patients to withstand the problems of sight-threatening eye diseases related to oxidative stress and accelerated biological aging in linked with earlier onset of diseases [171]. Can-C™ Plus is now available in US and European markets. Patents: (WO 2004/028536 A1; WO 94/19325; WO 95/12581; WO 2004/064866 A1).

Our findings and therapeutic concept that nonhydrolyzed carnosine-dependent redox homeostasis plays a pivotal role in the preservation of telomere integrity in senescent cells suggest that perturbation or depletion of this system may be an important mechanism for oxidative stress-induced premature senescence of the subjected cells and is entirely consistent with a role for telomere dysfunction in the pathogenesis of age-related disease. The provided finding that cellular aging can be bypassed or put on hold by the introduction of the telomerase catalytic reverse transcriptase cDNA is potentially very important from a basic research point of view. In addition, the medical implications of introducing telomerase into normal cells are profound.

However, more clinically oriented technology utilizing imidazole-containing dipeptides (nonhydrolyzed carnosine, carcinine) could allow a patient’s own cells to be removed, manipulated, and/or rejuvenated without using up their life span and then returned to the patient. The prospect that manipulating TL by using the therapeutics with nonhydrolyzed natural imidazole-containing compounds and patented formulations thereof may change the rate of cellular aging and affect the degenerative diseases of aging is truly an exciting possibility. Furthermore, the longitudinal studies of elderly individuals have suggested that longer telomeres are associated with better survival and an advanced oral nutritional support with nonhydrolyzed carnosine (or carcinine remedies) is a useful therapeutic tool of a critical TL maintenance that may fundamentally be applied in the treatment of age-related sight-threatening eye disorders, prolonged life expectancy, increased survival, and chronological age of an organism in health control, smoking behavior, and disease [7–10, 171].

Acknowledgments: This work was planned, organized, and supported by Innovative Vision Products, Inc. or IVP (New Castle County, DE, USA) by the Russian Foundation for Basic Research (grant project no. 13-04-01178) to Prof. Babizhayev et al.: Hormone-brain-aging behavior relationships, broadly reactive with carnosine. DE GRUYTER.
Y.E. Yegorov. IVP is a pharmaceutical and nanotechnology development company with a focus on innovative chemical entities, drug delivery systems, and unique medical devices to target specific biomedical applications. Over the last decade, IVP has developed a track record in developing these technologies to effectively address the unmet needs of specific diseased populations.

Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Research funding: None declared.
Employment or leadership: The author (Dr. Mark A. Babizhayev) reports the interest in the intellectual property of the described modalities protected with the patents. The authors bear primary responsibility for accuracy of made statements and employment of the described products and for the content and writing of the paper.

Honorarium: None declared.

References


