Open Access

OBM Geriatrics

LIDSEN Publishing Inc.

Review

Perspectives to Modify and Counter Aging in the Frame of Subtelomere–Telomere Theory of Aging

Giacinto Libertini [1, 2, *]

1. Member of the Italian Society for Evolutionary Biology (SIBE), 14100 Asti, Italy; E-Mail: giacinto.libertini@yahoo.com
2. External Collaborator of Department of Translational Medical Sciences, Federico II University of Naples, 80131, Naples, Italy

* **Correspondence:** Giacinto Libertini; E-Mail: giacinto.libertini@yahoo.com

Academic Editors: Kurt Whittemore and Michael Fossel

Special Issue: Perspectives on Telomeres and Aging II

OBM Geriatrics
2022, volume 6, issue 4
doi:10.21926/obm.geriatr.2204208

Received: July 22, 2022
Accepted: September 25, 2022
Published: October 14, 2022

Abstract

The interpretation of aging as an adaptive and programmed phenomenon implies the existence of specific genetically determined and regulated aging-causing mechanisms. This interpretation is in contrast to the explanation of aging as the gradual accumulation of the effects of harmful factors that are only partially countered by natural selection. The subtelomere–telomere theory of aging offers what is required by the interpretation of aging as a programmed phenomenon. The experimentally documented mechanisms that are part of the subtelomere–telomere theory are the repression of subtelomeric sequences (TERRA sequences) consequent to the sliding of a telomeric hood over subtelomere in proportion to telomere shortening, epigenetic modifications caused by the repression of the subtelomeric sequences, cell senescence and gradual cell senescence (which are not synonyms, as discussed in the text), progressive decline of stem cells, and effects of these phenomena over the whole organism. Evidence against the interpretation of cell senescence and telomerase restrictions as defense mechanisms against cancer is reported. Consequently, the fears that telomerase activation or senescent cell elimination are potentially oncogenic factors should be eliminated

as preconceived ideas or limited on the basis of any available evidence. In the context of the mechanisms described under the subtelomere–telomere theory, three types of strategies that could be used to modify and counter the mechanisms of aging can be deduced, namely telomerase activation, senescent cell elimination, and restoration of stem cell numbers to that existing in young individuals. The limits and the potential effectiveness of these methods, already the subject of active research, are briefly discussed.

Keywords
Aging; subtelomere; TERRA sequences; cell senescence; gradual cell senescence; stem cells

1. Introduction

Aging has two opposite explanations and is defined as:

- "a general title for the group of effects that, in various phyla, lead to a decreasing expectation of life with increasing age" [1], p. 7;
- "increasing mortality with increasing chronological age in populations in the wild" [2];
- "increasing mortality with age … actuarial senescence," in populations in the wild [3].

According to the first explanation, aging is gradually caused by the effects of one or more harmful factors that natural selection is only partially able to counter. Examples of harmful factors include the oxidative effects of free radicals on the whole body [4], mitochondria [5], and DNA [6]. Aging is a harmful phenomenon that cannot be programmed, that is, it cannot be caused by specific mechanisms determined and regulated by genes. Natural selection always tries, with limited effectiveness, to counter its causal factors and delay aging.

According to the second opposite explanation, aging is an adaptive phenomenon favored by selective mechanisms at the supra-individual level, and is a type of phenoptosis (programmed death of an organism) [7, 8] or programmed organismal death (POD) [9]. In several species, the life cycle is determined by forms of phenoptosis (e.g., in animals that reproduce or plants that bloom and die soon after [10]), which implies the existence of specific mechanisms determining them.

This work does not want to expound or discuss arguments and evidence in support or against the two explanations, a topic that can be deepened in other works (e.g., see [11] and [12], Chapter 4). Rather, the strategies to oppose aging are discussed here. However, to counter aging, the mechanisms underlying the phenomenon must be uncovered.

For this purpose, aging should be interpreted as a programmed phenomenon. The second explanation implies the existence of specific aging-causing mechanisms that are genetically determined and regulated. Such hypothetical mechanisms should be described and supported by scientific evidence.

First, the experimentally documented mechanisms that appear to determine aging must be discussed, thereby proving the second explanation and disowning the opposite explanation. On the basis of these mechanisms, a logical deduction of the strategies that could be used to modify and counter aging is possible.

2. The Subtelomere–Telomere Theory of Aging

A few studies explained the mechanisms underlying aging by proposing the subtelomere–telomere theory of aging [13, 14], which is a necessary transformation of the previous telomere theory of aging.

2.1 The Telomere Theory of Aging and Its Limitations

In 1961, a study demonstrated that cells could undergo only a limited number of duplications [15]. In 1971, Olovnikov observed that DNA replicating enzymes allow for partial duplication as a small terminal part is not duplicated. This results in the progressive shortening of the terminal part of DNA (telomere), which may inhibit cellular duplication [16]. To explain the unlimited duplications of germ line cells and stem cells, Olovnikov conducted a study 2 years later and stated that an enzyme with the ability to restore the nonduplicated part of the DNA should exist [17]. This enzyme, called telomerase, was isolated in 1985 [18].

Other studies have confirmed the relationship between telomere length, telomerase activity, and cell duplication capacity. In particular, they demonstrated that:

- In human fibroblasts, telomeres are shortened with increasing age and number of cell duplications [19];
- *Tetrahymena* mutant strains with inactive telomerase exhibited a reduced duplication capacity [20];
- human cell lines with an unlimited capacity for duplications exhibited nonrestricted activity of telomerase [21];
- telomerase activation enabled unlimited cellular duplication [22].

Cells can lose their duplication capacity, with cellular functions being stereotypically altered. This condition is defined as “cell senescence” and is related to telomere shortening, leading to the aging of tissues and organs [19, 22]. The condition was later described as a “fundamental cellular program” [23].

These empirical data led to the hypothesis that the effects of telomere shortening, namely restrictions in cell duplication capacities and in cell turnover and alterations caused by the increasing number of cells in cell senescence, were the cause of a progressive decay of the body’s functions. This hypothesis led to the proposal of the telomere theory of aging.

If this theory was true, it should predict a direct relationship between life span and initial telomere length (i.e., telomere mean length in germ line cells) and telomerase activity. However, this was contradicted by the following findings:

- compared with humans, hamsters and mice have longer telomeres and shorter life spans [24];
- somatic cells in mice have a baseline activity of telomerase [25];
- two *Mus* strains having different telomere lengths (10 kb and 20 kb) exhibited equal life span and increments of age-related mortality [26], p. 60;
- among the rodents, life span exhibited no relationship with telomere length [27] and telomerase activity [27, 28], whereas telomerase activity and body mass appeared related [27];
- cloned animals obtained from somatic cells exhibited patterns of aging that were similar to those of the donor animal, although the original cell of the cloned animal had shorter telomeres than the germ cell of the donor animal (see [29, 30] and [26], p. 60);

- in *Mus* strains with inactivated telomerase in protected laboratory conditions, viability and fertility were compromised only when telomeres were significantly shortened (i.e., after four [31] to six [32] generations); moreover, in previous generations, no obvious differences were observed despite the considerable difference in telomere lengths.

Furthermore, the hypothesis could not explain how tissues and organs with a prevalence of perennial cells, that is, cells without turnover and duplication capacity (e.g., the cerebral cortex and other parts of the central nervous system), aged like other tissues without perennial cells.

Because of these results, the telomere theory of aging was discredited. The shortening of telomeres improperly described, even in recent works, as telomere attrition, seemed to be another type of damage that accumulates over time in accordance with the first explanation of aging.

2.2 Preliminary Considerations for the Subtelomere–Telomere Theory of Aging

The aforementioned findings indicated that the telomere theory of aging either had to be radically modified or telomere shortening could be another harmful factor that explained aging as a nonadaptive phenomenon.

However, other empirical data had to be considered or were emerging:

(1) Blackburn observed [33] that the transition from a cell capable of duplication to a cell in the state of cell senescence was not activated only by a critical telomere shortening. However, the probability of cell senescence triggering was related to telomere shortening. The decline in the growth of cultures was not sudden after a certain number of duplications but was a progressive decline in duplication capacity [34, 35]. Blackburn proposed that the telomere was covered by a cap formed by particular molecules (sequence-specific DNA-binding proteins) and that the telomere and cap (DNA-protein complex) oscillated between the following states: (i) capped telomere, in which the telomere is protected, and the cell is resistant to cell senescence and (ii) uncapped telomere, in which the telomere is less protected, and the cell is vulnerable to cell senescence triggering. This implied that even cells with unshortened telomeres or with a small shortening had some probabilities of cell senescence activation. Furthermore, as Blackburn proposed, the bond between the telomere and cap weakened with telomere shortening, thereby indicating that a regulatory mechanism dependent on telomere length exists.

(2) In yeast, the insertion of a gene in a position close to the telomere determines its repression. "Yeast telomeres exert a position effect on the transcription of nearby genes, an effect that is under epigenetic control" [36]. This repression, defined as the "telomeric position effect" [36], was later demonstrated in humans [37] and other mammals [38]. Furthermore, a study on a rare human genetic disease (ring 17 syndrome) revealed that the telomere position effect is related to telomere shortening [39].

(3) In yeast, the telomeric position effect and aging were related:

- Wild strains of yeasts have permanently active telomerase; therefore, the telomeres do not shorten when there is a duplication [40, 41]. Each yeast cell divides into two slightly different cells, mother and daughter cells. The cells of the daughter lineage can divide an unlimited number of times [41], whereas the cells of the mother lineage can divide to a limited extent (25–35 duplications in approximately 3 days [42]). Moreover, in relation to the number of duplications, the cells of the mother lineage exhibit (i) a progressive

accumulation of particular molecules, called extrachromosomal ribosomal DNA circles (ERCs), on DNA immediately adjacent to the telomere (defined as subtelomere) [43]; (ii) progressive functional alterations; and (iii) an increasing probability of replicative capacity loss soon followed by apoptosis [44-48].

- Yeast *tlc1Δ* mutants have a deficient telomerase activity and, therefore, both mother and daughter cells exhibit telomere shortening at each duplication. Compared with mother cells of the wild strain, cells of the daughter lineage (without ERC accumulation as for wild strains) with the same number of previous duplications exhibit reduced resistance to stress and a similar transcriptome to mother cells [45]. The metabolic alterations exhibited by the mother cells of wild strains and by the daughter cells of *tlc1Δ* mutant strains could be a consequence of subtelomere repression, which in turn causes alterations in other parts of the DNA. Subtelomere repression and its effects in the mother cells are determined by ERC accumulation [43], whereas in the daughter cells without ERC accumulation, it could be caused by the sliding of a telomere cap over the subtelomere due to the progressive shortening of the telomere [26, 49].

(4) While comparing different species, in addition to the differences in telomere length (which is not related to life span), the following factors must be considered: (i) several telomeres exist in a single cell (two telomeres for each DNA molecule, with two copies in each chromosome; to obtain the number of telomeres, the number of chromosomes should be multiplied by 4, i.e., in humans with 23 chromosomes, the number of telomeres = 23 × 4 = 92); (ii) the initial telomere length (i.e., that of the germ cell) of different telomeres, even in the same DNA molecule, is not equal "... telomere lengths within the same cell are heterogeneous and certain chromosome arms typically have either short or long telomeres." [50]; and (iii) the differences in telomere length are hereditary, and similar telomere lengths are observed in monozygotic twins but not in dizygotic twins [51, 52].

The variability and poor phylogenetic stability of the telomere length in the course of evolution are in contrast to the extreme phylogenetic stability of the telomeric sequence. A study on a protozoan species revealed that each telomere was a simple sequence of nucleotides (motif) repeated many times (TTG GGG) [53]. Some years later, another study discovered that mammalian telomeres had a motif with a little difference (TTAGGG) [54] and that this sequence was the same in trypanosomes, molds, and other nonmammal vertebrates and organisms [55]. The Telomeric Sequence Database [56] demonstrates that the mammalian motif is shared by all vertebrates and also by phylogenetically distant species (e.g., *Nicotiana tabacum*, common tobacco) and that another similar motif (TTAGG) is present in various phylogenetically distant species (e.g., *Apis mellifera*, honey bee, and *Giardia lamblia*, a protozoan parasite). The extraordinary conservation of the motifs, even among species with distant ancestors, indicated the importance of the telomeric structure. However, data on the relationship between the telomere and aging did not indicate the importance of the initial length of the telomere.

2.3 General Description of Subtelomere–Telomere Theory of Aging

To solve the incongruities of the telomere theory of aging, some studies suggested the fundamental role of the subtelomere, that is, the portion of DNA that follows the telomere [26, 57].

This proposal was subsequently deepened and called the subtelomere–telomere theory of aging [13, 14, 58, 59].

The subtelomere–telomere theory of aging was proposed based on the aforementioned findings (1–4) and on others not mentioned here for the sake of brevity. Its central idea was expressed by Fossel: "... a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome... As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) ... the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity... These silenced genes may, in turn, modulate other more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [26], p. 50.

In short, the subtelomere–telomere theory of aging proposed the following:

(i) Each telomere is covered by a telomeric cap (or hood), with a size defined in the first cell of the organism and not following a fixed size but adapting to the length of the telomere;

(ii) The size of each telomere does not vary in any subsequent duplication, even if the telomere shortens due to insufficient or zero telomerase activity;

(iii) As the telomere shortens, the hood becomes longer than the telomere, and the excess part inhibits a growing part of the subtelomere in which there are hypothetical regulatory sequences, defined as r-sequences. This was also described as the sliding of the hood over the subtelomere. In yeast cells of the mother lineage, the subtelomere repression was determined by the progressive accumulation of ERCs, whereas in the cells of the daughter line with the *tlc1Δ* mutation, the subtelomere repression appeared to exhibit the same mechanism as described before;

(iv) the aforementioned r-sequences were also hypothesized to have direct or mediated regulatory effects on other regulatory sequences and their repression caused a general alteration in cell functions;

(v) the consequences of the repression of the r-sequences included (a) gradual alterations of cellular function defined as gradual cell senescence; and (b) reduction of the telomere–subtelomeric hood complex stability with the consequent increasing probability of activation of the cell senescence program, leading to an increased number of cells in replicative senescence.

The subtelomere–telomere theory overcame the following drawbacks of the telomere theory:

- Even though the telomeres varied in length, the cap was modeled on the initial telomere length. The subsequent subtelomere repression depended on telomere shortening and not on the initial absolute lengths of the telomeres. Consequently, a correlation between initial (mean) telomere length and life span was not predicted.
- The activation of the cell senescence program was not a function of substantial telomere shortening but depended on the progressive repression of the subtelomere, thereby influencing the telomere–telomeric hood complex stability. This indicated the probability of cell senescence program activation in accordance with that envisaged by Blackburn [33].
- In cascade, all the manifestations of aging depended on these phenomena, as explained in detail in the following sections.

If the life span is not related to the initial telomere length but to their subsequent shortenings, how the considerable difference in life spans among various species originates should be determined. Various factors may regulate life span, such as different (i) subtelomeric structure; (ii) telomerase repression; and (iii) telomere shortening rate (an inverse relationship between life span and telomere shortening rate was demonstrated [60]).

2.4 TERRA Sequences

The subtelomere–telomere theory, despite overcoming the difficulties of the telomere theory, had a major weakness. As long as r-sequences and their particular regulatory capabilities were only a hypothesis not confirmed by evidence, the whole construction of the theory could prove to be unfounded.

In the unawareness of those who proposed the subtelomere–telomere theory of aging, sequences with the precise characteristics hypothesized for the r-sequences were already known and studied by talented researchers, who in turn, not knowing the subtelomere–telomere theory, were not aware of the importance of their work in elucidating the mechanisms underlying aging.
In 1990, two subtelomeric sequences (TelBam3.4 and TelSau2.0) [61] with conserved regions of 1.6 and 1.3 kb long, respectively, were described [62].

The two sequences defined (perhaps in a partially misleading way as they presuppose a delimitation of the telomere extended to the adjacent section) as telomeric repeat-containing RNA or TERRA (for brevity, "T-sequences") (i) are subject to transcription and produce RNA sequences (for brevity, "T-transcripts"); (ii) do not code for proteins; (iii) are present in humans [63, 64], plants [65], zebrafish and mouse [64], and yeast [66].

Moreover,

- T-sequences are "evolutionarily conserved in vertebrates" [67], are a general characteristic in eukaryotic cells, and "are emerging as new key players in several important biological processes" [68]. Therefore, T-sequences have a pivotal function from ancestral times;
- The transcription of T-sequences, mediated by the enzyme RNA polymerase II, begins from subtelomeric promoters located on at least two-thirds of chromosome ends [62, 69, 70] and proceeds toward the repeated motif of the telomere by including some of them in the transcription [63, 64, 71];
- "The vast majority of TERRA-binding sites were found outside of telomeres, mostly in distal intergenic and intronic regions of the genome where TERRA regulates gene expression" [68]. T-transcripts bind to many loci with noncoding DNA sequences that have important regulatory functions in gene expression [72, 73];
- "The first human subtelomeric promoters that were identified comprise CpG dinucleotide-rich DNA islands shared among multiple chromosome ends ..." [68];
- "TERRA read coverage was high within subtelomeric regions of nearly all chromosomes ... with targets being as much as tens of kilobases away from the telomeric repeat ... TERRA also bound within internal chromosomal regions and within genes, where it favored introns ... TERRA binds chromatin targets throughout the genome. ... TERRA binds both in cis at telomeres and in trans within or near genes." [72];
- Evidence indicates "... significant changes in expression of TERRA targets relative to nontargets after TERRA depletion ..., indicating that TERRA target genes were more likely to

be affected by TERRA depletion. ... Interestingly, subtelomeric target genes were consistently downregulated ... Internal target genes could either be up- or down-regulated ... In the mouse ES [embryonic stem] cell genome, we identified thousands of cis and trans chromatin binding sites" [72];

- "Cycling endurance exercise, which is associated with AMPK activation, increased TERRA levels in skeletal muscle biopsies obtained from 10 healthy young volunteers. The data support the idea that exercise may protect against aging." [70];
- The blockage of T-sequences is related to "defects in the capping function. With telomere-specific probes, DNA FISH analysis of metaphase spreads revealed loss of telomeric integrity after 24 h TERRA knockdown ..." [72]. In mice, the depletion of T-transcripts in embryonic stem cells is related to reduced telomere protection [72, 73];
- The inhibition of T-sequence transcription triggers a DNA damage response at telomeres [74]. The deletion of the 20q locus leads to a collapse of T-transcripts, followed by a massive DNA damage response, which appears to be a "demonstration in any organism of the essential role of TERRA in the maintenance of telomeres" [75];
- T-transcripts are antagonists of ATRX, which is a protein related to alpha thalassemia mental retardation X-related syndrome and are essential for telomere protection. "TERRA and ATRX share hundreds of target genes and are functionally antagonistic at these loci. Whereas TERRA activates, ATRX represses gene expression. At telomeres, TERRA competes with telomeric DNA for ATRX binding, suppresses ATRX localization, and ensures telomeric stability." [72].

These experimental data indicate that T-sequences have the features hypothesized by the subtelomere–telomere theory for r-sequences. In particular, T-sequences

- are located in the subtelomeres;
- are widespread even in phylogenetically distant species, appear evolutionarily conserved, and perform functions of pivotal importance;
- are increasingly repressed with telomere shortening;
- produce transcripts that regulate other regulatory sequences located at their immediate vicinity and at other parts, even at distant ones, of the DNA molecule in which they are found or of other DNA molecules of the same cell;
- regulate innumerable parts of the genome, directly or indirectly, thereby influencing several cellular functions in various ways;
- are essential for the stability of the telomere and the telomere-telomeric hood complex, thereby activating the cell senescence program.

Consequently, in the subtelomere–telomere theory, the replacement of the hypothetical r-sequences with the T-sequences appears correct.

This confirms the existence of a mechanism that progressively alters cellular functions and, consequently, affects the overall functions of the organism (i.e., determines aging). This supports the explanation of aging as an adaptive and programmed phenomenon and contradicts the explanation of nonadaptive aging. To defend the first explanation, we should explain the presence of T-sequences at a position that is most vulnerable to the effects of telomere shortening, i.e, the telomeric position effect.

2.5 Age-Related Epigenetic Modifications and Their Relationships With T-Sequences

Following are the age-related epigenetic modifications (EMs) of DNA:

- Age and EMs, which vary according to the type of cells and tissues, are strongly related [76, 77]. Cytosine-5 methylation within CpG dinucleotides (DNA methylation) is the most studied age-related EM [78, 79];
- In embryonic cells and induced pluripotent stem cells (iPSCs), DNA methylation is practically inexistent; however, its frequency increases in proportion to the number of cell duplications [78, 79].
- In our species, the frequency of DNA methylation is an indicator of age, with a correlation value with age equal to 0.96 and an error of 3.6 years [78];
- In a study based on approximately 128 mammalian species (with life spans between 3.8 and 211 years and a similar range of variation in adult weight), an analogous indicator was proposed with a correlation greater than 0.96 and a median relative error less than 3.5% [79];
- In a study, the reversibility of age-related EMs was demonstrated by the transformation of adult somatic cells into iPSCs [78];
- Age-related DNA methylation in CpG sequences [80-83] is limited to particular parts of DNA molecules, defined as CpG islands (CGIs), where the frequency of CpG nucleotides is 1 every 10 bp. CGIs constitute approximately 2% of the entire DNA [76] and often coincide with the transcription start sites [84];
- The methylation of CGIs is correlated with the silencing of promoters present in them [85], whereas demethylation restores promoter expression [86];
- Age-related DNA methylation of CGIs can be either hypomethylation or hypermethylation [82, 83, 87, 88];
- In general, CGIs are evolutionarily conserved, can be used as an index to assess age that is reliable and valid for mammals in general [79];
- DNA methylation is not the only known age-related EM (other age-related EMs include “reduced bulk levels of the core histones, altered patterns of histone posttranslational modifications ..., replacement of canonical histones with histone variants, and altered noncoding RNA expression” [89], nucleosome remodeling, histone methylation, changes in histone marks, reduction of heterochromatin [90, 91]). However, the two best EM-based indicators for assessing age are the ones mentioned before regarding DNA methylation [78, 79].

Following are the links between age-related EMs and the effects of T-sequences:

- The CGIs located in the subtelomere “promote transcription of TERRA molecules.” [62];
- “Subtelomeric DNA methylation is ... decreased in conjunction with telomere shortening in Terc (-/-) mice.” [92];
- In mice, telomere shortening is correlated with subtelomere methylation [93]. “Furthermore, the abrogation of master epigenetic regulators ... correlates with loss of telomere-length control, and telomere shortening to a critical length affects the epigenetic status of telomeres and subtelomeres.” [93];
- A study demonstrated an association between aging and an increasing number of short telomeres with hypomethylated subtelomeres in healthy individuals and patients with

sarcoidosis; however, in younger patients, long telomeres with hypermethylated subtelomeres were more frequent [94];

- In human leukocytes, "... shorter telomeres are associated with decreased methylation levels of multiple cytosine sites located within 4 Mb of telomeres ... significant enrichment of positively associated methylated CpG sites in subtelomeric loci (within 4 Mb of the telomere) ($P < 0.01$)" [95]. Telomere shortening is related to gene expression modifications and an increased risk of various age-related diseases [95];
- Cell senescence in mesenchymal stem cells (MSCs) is related to some markers of aging, such as DNA methylation in specific CGIs and trimethylation at particular histone targets [96];
- In the same type of cells, "expansion of MSC has a very consistent impact on DNA-methylation profiles;" "517 CpG sites were consistently differentially methylated in early versus late passages" [96];
- In MSCs, after various duplications, hypermethylation is observed in some CpG sites, whereas hypomethylation is observed in others. "Almost one-third of the CpG sites reveal age-associated changes in DNA methylation, of which 60% become hypomethylated and 40% hypermethylated upon aging." [97].

These data suggest clear correlations between T-sequences and a series of EMs, and it is unlikely that these EMs are determined by random factors. Therefore, cellular aging, and the consequent general aging of the organism, is an epigenetic phenomenon caused and regulated by the repression of T-sequences. However, the definition of aging as a genetically determined and regulated epigenetic phenomenon should not be considered a peculiarity of aging but may be applicable to several other functions of the organism.

The Human Genome and ENCODE projects demonstrated that "the protein-coding potential of the mammalian genome is extremely limited ... Although only 2% of the genome is coding, >90% is transcribed. This transcriptional activity largely produces long noncoding RNAs (lncRNA), the functions of which have remained mostly unknown." [72]. The number of proteins encoded by genes does not vary much in relation to the complexity of a species. For example, the number of protein-coding genes in humans and a simple nematode is nearly equal [98].

Therefore, the programs that define any function, physiological organization, and morphological development of an organism are mostly not located in protein-coding DNA but in the much longer DNA parts that regulate the whole DNA, including the protein-coding sections. The functions of an organism probably occur by activating or repressing actions obtained through EMs, and aging is not an exception to this general rule.

This is consistent with the explanation of aging as a programmed phenomenon and contradicts the opposite explanation of aging as a consequence of the accumulation of harmful random events.

For completeness, it is appropriate to add the following considerations:

- The two subtelomeric sequences, TelBam3.4 and TelSau2.0, do not exclude the existence of other subtelomeric sequences with similar characteristics (e.g., the telomeric damage-induced long ncRNAs (tdilncRNA) [99]);
- Telomere alterations are noted in particular genetic diseases that, at least in part, exhibit characteristics similar to those of physiological aging (progeroid syndromes, PSs). For example, in laminopathies (e.g., Hutchinson-Gilford Progeria Syndrome) with malfunctions of the LMNA gene, cell duplication alterations, telomere shortening, and genomic instability are observed [100]. A detailed discussion of PSs is beyond the scope of this review. However, a detailed

discussion on the mechanisms and manifestations of two PSs, Werner syndrome (adult progeria) and Dyskeratosis congenita, in relation to the subtelomere–telomere theory of aging is offered in Section 7.4 (Disease due to genetic alteration that causes aging-like syndromes) of a recent book [12].

2.6 Gradual Cell Senescence

According to subtelomere–telomere theory, telomere shortening causes progressive subtelomeric inhibition with increasing alterations of cellular functions, a phenomenon defined as gradual cell senescence [11]; and increasing probability of cell senescence activation, which determines both replicative senescence and pronounced alterations of cellular functionality.

In cell culture, and also in a progressively aging tissue or organ, both cells in the gradual cell senescence state and cells in the cell senescence state are present. However, how much of the overall functional alterations are due to one or the other phenomenon remains uncertain. There is a tendency to confuse the cells in the gradual cell senescence state with those in the cell senescence state as if it were the same phenomenon in different degrees. “There is substantial variability in the degree of senescence and few if any fully senescent cells, but a significant degree of altered gene expression within a percentage of partially senescent cells.” [26], p. 148.

Gradual cell senescence is little known or is confused with cell senescence despite the existence of the following empirical data that clearly support the distinct existence of gradual cell senescence:

- A culture of duplicating MSCs exhibited gradual changes in mRNA expression, with “a consistent pattern of alterations in the global gene expression ... These changes are not restricted to later passages but are continuously acquired with increasing passages” [101]. Furthermore, concerning the number of previous duplications, MSCs exhibit gradual changes in DNA methylation, whose measurement can be used to calculate the number of duplications [102-104];
- In a study investigating the consequences of telomere shortening, the authors stated that, “Our results demonstrate that the expression of a subset of subtelomeric genes is dependent on the length of telomeres and that widespread changes in gene expression are induced by telomere shortening long before telomeres become rate-limiting for division or before short telomeres initiate DNA damage signaling. These changes include upregulation and downregulation of gene expression levels” [105].
- In cultures of yeast, a unicellular organism where cell senescence causes immediate apoptosis [44, 46], cells of the mother lineage of wild strains, in which the subtelomeric repression is caused by ERC accumulation, exhibit increasing functional alterations and susceptibility to cell senescence in proportion to the number of previous duplications [44, 46]. Moreover, cells of the daughter lineage with the *tlc1Δ* mutation exhibit inactive telomerase and shortened telomeres with each duplication. Due to the progressive subtelomeric inhibition (in the absence of ERC accumulation), in proportion to the number of previous duplications, these cells exhibit functional alterations and a transcriptome similar to that of cells of the mother lineage of wild strains with the same number of duplications [45].

These phenomena cannot be attributed to the casual accumulation of harmful substances or to other factors that act randomly. This possibility is contradicted by the following experiments that demonstrated the complete reversibility of functional alterations:

- The increasing functional alterations observed in MSCs in proportion to the number of previous duplications are canceled by the reprogramming of these cells in iPSCs [106]. These iPSCs, irrespective of the source of the cell and the age of the donor, exhibited the profile of a young cell [106].
- In induced MSCs (iMSCs), "DNA methylation, related to age, was completely erased, and iMSCs reacquired senescence-associated DNA methylation during culture in vitro" [97].
- iMSCs can be derived from iPSCs and exhibit superior cell functions and fewer epigenetic modifications [107].

Because gradual cell senescence reduces the functional efficiency of the cell and, therefore, of the entire organism to which the cell belongs (in the case of a multicellular organism), the phenomenon should be eliminated by natural selection at the individual level. For its existence, the action of selective mechanisms at a supra-individual level appears necessary, which is perfectly justifiable in the context of a general program aimed at the progressive reduction of survival probabilities, as proposed by the explanation of aging as an adaptive and programmed phenomenon. However, the nonadaptive explanation of aging requires a justification for gradual cell senescence.

2.7 Cell Senescence

A cell in the cell senescence state (senescent cell) is not an old cell but a cell in which a specific cellular program [23] has been activated by damaging factors (e.g., altered culture conditions, oxidative stress, and DNA damage [108, 109]). Moreover, the probability of the program being triggered is related to telomere shortening [33, 110].

A senescent cell is characterized by the following pattern of cellular alterations:

(i) replicative senescence [111, 112];
(ii) resistance to apoptosis [113, 114];
(iii) stereotyped alterations of cellular functions [112, 114, 115], with "profound transcriptional changes" [116]; and
(iv) stereotyped alterations of extracellular secretions, known as senescence-associated secretory phenotype (SASP) [117, 118].

The senescent cells, identified on the basis of $p16^{Ink4a}$ expression, increase in relation to age both as a fraction of the total number of cells and in their absolute number [119, 120].

This increase is related to the extent of aging and age-related diseases [121, 122]. Selective elimination of senescent cells reduces these manifestations [122, 123]. Drugs with this effect are known as senolytics and can be used to counter the manifestations of both aging and age-related diseases [114, 123-125].

In the context of the subtelomere–telomere theory and the explanation of aging as an adaptive and programmed phenomenon, senescent cells play an effective role in progressively reducing the efficiency of the organism and, therefore, the ability to survive, i.e., aging. In the context of the first explanation, namely the interpretation of aging as the causal accumulation of damage resulting from multiple factors, the phenomenon of cell senescence requires a specific plausible justification.

The only explanation proposed justifies cell senescence as a general defense against uncontrolled cell proliferation in cancer because cell senescence determines, among other things, the blockage of duplication capacity [126, 127]. Furthermore, since cell senescence, in addition to the hypothesized action against tumor proliferation, certainly causes various harmful effects, this has

been justified as an example of antagonistic pleiotropy, that is, a case in which positive and negative effects are clashing in natural selection [128].

The following findings make this justification doubtful or untenable:

- As part of the altered secretions constituting the SASP, there are "myriad factors associated with inflammation and malignancy" [117];
- In mice, selective elimination of senescent cells led to a delayed progression of induced cancer and age and increased life span [120];
- In humans, a relationship between cancer risk and short telomeres (which increase the probability of cell senescence) has been observed [129, 130];
- In mice, induced telomerase expression did not increase the risk of cancer, delayed aging manifestations, and increased life span [131];
- "Senescent cells are present in premalignant lesions and sites of tissue damage and accumulate in tissues with age" [132];
- The authors of a study [133] declared that "cellular senescence suppresses cancer by irreversibly arresting cell proliferation", but observed that in cancer therapies, "several chemotherapeutic drugs induce [cell] senescence" and "eliminating TIS [therapy-induced senescent] cells reduced several short- and long-term effects of the drugs, including ... cancer recurrence ..."
- The authors of another work [134] declared that cell senescence is a "potent cancer-protective response to oncogenic events", but proposed a model in which cell senescence is related to "an inflammatory phenotype and cancer."
- In yeast, cell senescence determines immediate apoptosis [135]. Because cancer does not occur in a monocellular species, cell senescence cannot have any anticancer significance for species like yeast.
- Other facts and arguments against this justification have been discussed in some studies [59, 136].

An outline of the subtelomere–telomere theory of aging is illustrated in Figure 1.

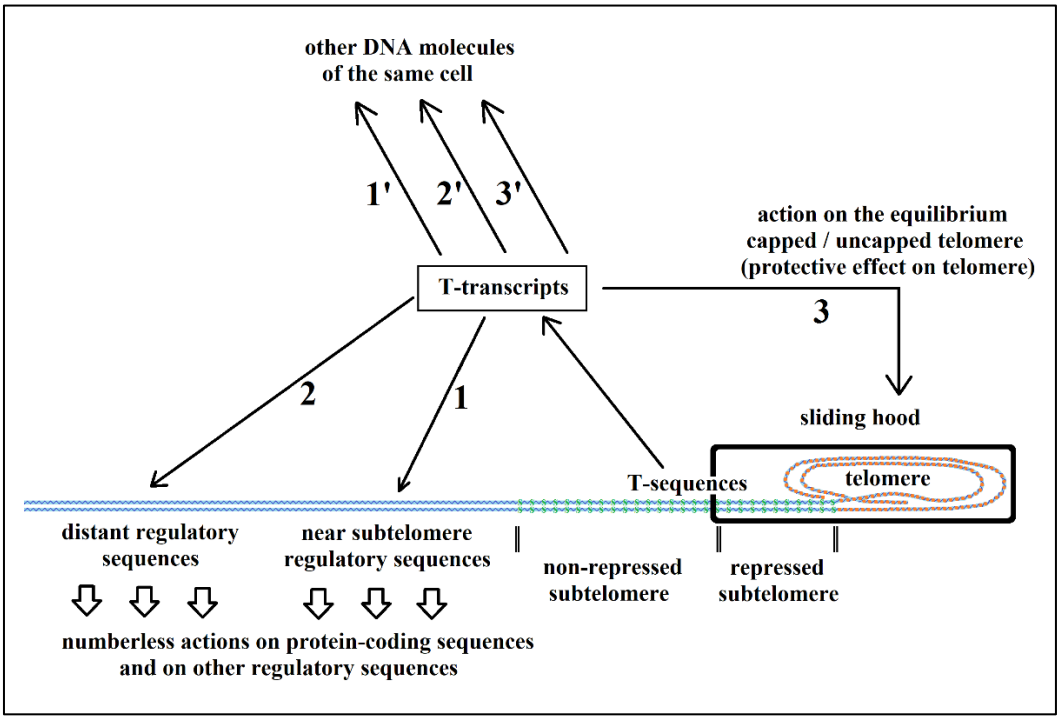


Figure 1 Schematic of the subtelomere–telomere theory. The T-sequences, which are progressively repressed by telomere shortening and the consequent sliding of the hood over the subtelomere, act through their transcripts (T-transcripts) (1) on nearby regulatory subtelomeric sequences; (2) on regulatory sequences in other parts of the chromosome; and (3) on the equilibrium between capped and uncapped conditions of the telomere. The T-transcripts have analogous actions on other DNA molecules of the same cell (1', 2', and 3'). The actions 1, 1', 2, and 2' determine modifications of the regulations of numberless genes and other regulatory sequences and, therefore, cause progressive alterations of cellular functions (gradual cell senescence). Actions 3 and 3' reduce the vulnerability of the cell to the triggering of the cell senescence program (replicative senescence + stereotypical alterations of cellular functions + resistance to apoptosis).

2.8 The Telomeric Heterochromatin Hood

The heterochromatin hood or cap that covers and protects the telomere is discussed in detail as an important element of the subtelomere–telomere theory in a study [14]. To avoid repetitions, only an essential point will be mentioned here.

The telomeric hood is formed by the coverage of the telomere by several copies of the shelterin protein complex. Both the main components (proteins TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 [137, 138]) of the complex and the likely arrangement of these proteins in the complex is well known [137].

According to the subtelomere–telomere theory, the size of the telomeric hood must be determined in the first cell of the organism on the basis of the length of the telomere, which varies from telomere to telomere even in the same cell. Moreover, this size must not vary in subsequent cell duplications, even when the telomere is shortened.

If the theory is true, a possible prediction is that the cellular amount of shelterin proteins should not be related to the total length of the telomeres, which vary in relation to the number of duplications, but should be constant. On the contrary, if the size of the hood changes in proportion to telomere shortening, a reduction in the number of shelterin proteins proportional to the shortening is expected.

A study supported the invariability of the telomeric hood size by stating that "we used quantitative immunoblotting to determine the abundance and stoichiometry of the shelterin proteins in the chromatin-bound protein fraction of human cells. The abundance of shelterin components was similar in primary and transformed cells and was not correlated with telomere length" [139].

2.9 Cell Turnover and the Limits Determined by Aging

Our body is prevalently made up of cells that are continuously replaced. Cell death is either caused by accident-induced necrosis or by genetically regulated self-destructive mechanisms known as programmed cell death (PCD). Some cell turnovers determined by PCD have been known for a long time [140] (e.g., cells at the top of intestinal villi detach and are substituted with cells derived from intestinal stem cells that reside in the crypts). Apoptosis, an important type of PCD, is an ordered process that does not cause inflammation and releases cell parts that can be used by other cells [141]. Apoptosis is also exhibited by unicellular eukaryotic species (e.g., *Saccharomyces cerevisiae* [142]); moreover, it allows cellular turnover in different cell types (e.g., gliocytes [34], hepatocytes [143], and chondrocytes [144]).

The rate of cell turnover varies according to the cell type [145], with the turnover rate being low for some cell types (e.g., approximately 4.5 years for cardiac myocytes [146] and 10 years for bone [147]); however, for intestinal epithelium, the rate is very rapid, i.e., 3–6 days [147]).

A simplistic interpretation of the subtelomere–telomere theory would indicate that actively reproducing stem cells should originate cells with increasingly shortened telomeres in proportion to the speed of turnover. Therefore, an aged organism should have critically short telomeres in most of its cells. The experimental data from the following studies indicate a complex situation.

- A review on the reduction of telomere length over the years in humans [148] concluded an annual reduction in many cell types. However, only a few cell types exhibited a substantial reduction in telomere length. For example, hepatocytes exhibit a yearly reduction rate of 120 bp/year, leading to a change in telomere length from 13.7 ±2.5 kbp in neonates to 8.7 ±1.4 kbp in centenarians. The telomere length reduction rates are less in most cell types, i.e., within 20–60 bp/year; however, in cell types with minimal or no turnover (e.g., myocardiocytes and neurons of the cerebral cortex), no detectable reduction in telomere length is observed [148]. Overall, this appeared in contrast with the homogeneity of aging in the whole organism.
- Telomere lengths are similar in different cell types of the fetus [149].
- The telomere shortening rates were similar in four types of cells and tissues (subcutaneous fat, leukocytes, skin, and skeletal muscle), although the cell turnover rates were different [150].
- In hematopoietic stem cells, telomere length was shorter than that of somatic cells in tissues with low turnover [150].

A satisfactory explanation was proposed for these contradictory results. In the first phase (expansive phase), in the growth period, the progenitor cells actively proliferate, giving rise to second-level stem cells in proportion to the subsequent necessary rates of cell turnover (for example, high rates for hematopoietic cells and minimal rate for myocardiocytes). This corresponds to a reduction in telomere length that is proportional to the degree of expansion. Subsequently, the second-level stem cells give rise to somatic cells with a relatively constant reduction of telomeres [150].

The decline in cell turnover is attributable to the vulnerability of stem cells to cell senescence, wherein replication is blocked, and not to the shortening of telomeres [33]. This gradually depletes the pool of stem cells and slows down the cell turnover rate.

The explanation of the aging of tissues/organs with no turnover of major cells was provided by two works [151, 152]. A detailed discussion and the necessary references are also provided.

In short, the viability of perennial cells depends on other cells that are subject to turnover. The decline of these satellite cells causes the dependent cells to decline and then die. The best-documented example is that of the photoreceptors of the retina, which are highly differentiated types of neurons. The viability of photoreceptors depends on the cells of the retinal pigmented epithelium, a highly differentiated type of gliocyte that is subject to turnover. The decline of these cells causes functional alterations and death of photoreceptors, leading to retinal aging and age-related macular degeneration. Similarly, the decline of specialized gliocytes (microglia cells, oligodendrocytes, and astrocytes), which serve neurons and their axons, causes neuronal death. This results in the aging of nervous tissue and disorders, such as Alzheimer's and Parkinson's disease.

Another perennial structure that depends on cells with turnover is the crystalline lens, which for its trophism depends on the cells of the lens epithelium. The decline of these cells results in cataracts. In contrast, not all neurons are perennial. For example, olfactory receptor cells, a specialized type of neurons, are subject to turnover and age, similar to other cells with turnover [151].

2.10 The Atrophic Syndrome

The aforementioned phenomena (age-related epigenetic modifications, gradual cell senescence, cell senescence, and decline of cell turnover) exert the effect of an atrophic syndrome on either the cells with turnover (direct aging) or on the cells or structures without a turnover but which are dependent on other cells with turnover ("indirect" aging) [12, 49, 153]. In short, the atrophic syndrome is characterized by the following:

- increase in the fraction of cells in the gradual cell senescence state, which is more or less accentuated depending on the degree of telomere shortening;
- increase in the fraction of cells in cell senescence, that is, cells with modified cellular functions, cell secretions altered according to a specific pattern (SASP), and replicative senescence;
- reduction in the cell turnover rate due to the progressive decline of the stem cells;
- the declining cell turnover leads to a reduction in the number of specific cells of a tissue, hypertrophy of the remaining specific cells, and replacement of specific cells with nonspecific cells;
- as a consequence of the decline of specific tissue cells, the SASP of senescent cells and the altered functions of cells in gradual cell senescence leads to alterations in the intercellular fluid and in the cells that depend on the functionality of the senescent or missing cells;

- decline and alterations of perennial cells occur as a consequence of the decline and functional alterations of the cells on which they depend;
- The aforementioned progressive alterations of cells and tissues cause anatomical and functional alterations of the organs and the overall functions of the organism;
- The reduction of telomere length causes possible telomere instability and increased risk of cancer [154, 155]. Cancer risk and cancer are possible consequences of telomere shortening and not an evolutionary justification for telomere shortening or aging.

These alterations, expounded in detail in [12] Chapter 6 (Aging in the human species), are summarized in Figure 2.

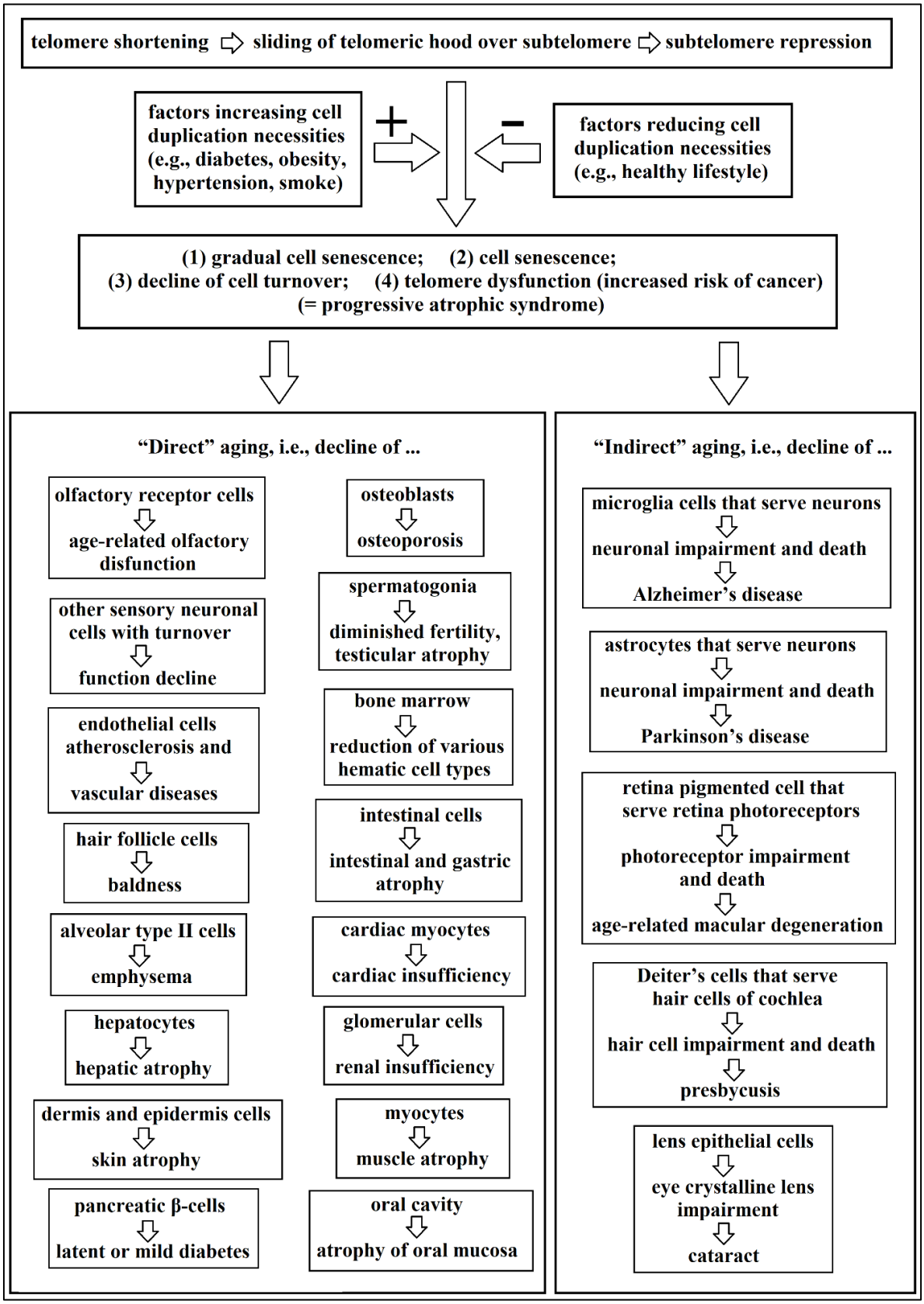


Figure 2 Schematic of the alterations produced by the atrophic syndrome in various organs and tissues, leading to a progressive decline in survival capacity, i.e., aging. Direct and indirect aging are presented separately (see text).

2.11 Distinction Between Aging and Age-Related Pathologies

The physiological process of aging and diseases caused by other factors (for example, smoking and excess salt, calorie, or fats in the diet) should be distinguished. Because these diseases progress with age, they are defined as age-related diseases and are often confused with the aging process. Therefore, the remedies adopted to combat these diseases are described improperly as anti-aging cures. However, these diseases often constitute an acceleration and aggravation of the physiological process of aging. Therefore, the methods used for their treatment should be described as cures against the pathological acceleration of aging and not as anti-aging therapies.

A study [156] demonstrated that endothelial cells originate from the duplication of the endothelial progenitor cells (EPCs) of the bone marrow. Furthermore, the number of EPCs was negatively correlated with age and various known risk factors for cardiovascular diseases (diabetes, hypertension, smoking, hypertension, overweight, and obesity). The decline in the number of EPCs and the so-called Framingham risk score [157] had an equal predictive value for the evaluation of cardiovascular risk.

The authors suggested that "continuous endothelial damage or dysfunction leads to an eventual depletion or exhaustion of a presumed finite supply of endothelial progenitor cell ... continuous risk-factor-induced injury may lead to eventual depletion of circulating endothelial cells" [156].

The study demonstrated a gradual decline of endothelial cells in physiological aging and this is the likely cause of cardiovascular diseases at older ages. The decline of endothelial cells, together with the pathological consequences, is accentuated by various risk factors and limited by some drugs. A careful review of the scientific literature indicated parallelism between the characteristic changes of aging and the decline of endothelial cells. Risk factors and drugs protective of endothelial cell function, such as statins, angiotensin-converting-enzyme inhibitors (ACE-i), sartans, or angiotensin II receptor blockers (ARBs), were also risk factors and protective drugs for other alterations of aging [12] (Table 1).

Table 1 Relationships between aging dysfunctions and the harmful effect of risk factors or the beneficial effects of protective drugs. This table has been appropriately modified from Tables 7.8 and 8.2 in a study [12]. The references (approximately 400) are reported in the same study and are omitted here for the sake of brevity.

	Effect on the risk by:							Protective effect by:	
Dysfunctions in the elderly	Age	Hypertension	Diabetes	Smoking	Obesity/ dyslipidemia	Moderate alcohol use	Alcohol abuse	Statins	ACE-i/ARBs
Endothelial dysfunction	+	+	+	+	+	-	+	+	+
Alopecia	+	+	+	+	+	+?	+	.	.
Atrophy of oral mucosa and salivary glands	+	+?	+	+	.	.	+	.	.
Atrophy of other sensory neuronal cells with turnover	+	.	+	+	.	.	+	.	.
Cardiac insufficiency and related diseases	+	+	+	+	+	-	+	+	+
Diabetes and impairment of glucose tolerance	+	+		+	+	-	+	-/	+
Emphysema and related diseases	+	+	+	+	-	-	+	+	+
Hepatic atrophy and related diseases	+	.	+	+	+	.	+	-/	+
Intestinal and gastric atrophy	+	.	.	+	.	.	.	.	.
Muscle atrophy	+	.	+	+	+	.	+	-	+
Olfactory dysfunction	+	+	+	+	+	.	+	+	.
Osteoporosis	+	+	+	+	+	-	+	+	+
Renal insufficiency	+	+	+	+	+	-	+	+	+
Skin atrophy	+	.	+	.	.	.	.	+	+
Testicular atrophy	+	.	+	+	+	/	+	.	/

Age-related macular degeneration*	+	+	+	+	+	-/	+	?	-?
Alzheimer's disease*	+	+	+	+	+	-	+	+	+
Cataract*	+	+	+	+	+	-	+	+?	+
Hearing impairment*	+	+	+	+	+	-	+	+	+
Parkinson's disease*	+	+/	+	-	+	-	+	+	+

Notes:

+ = increased risk or protective effect;

- = decreased risk or protective effect;

/= unaltered risk or protective effect;

? = unconfirmed results;

. = no specific study identified;

* = cases of indirect aging.

This parallelism suggests a substantial uniformity in the manifestations of the aging process and the possible pharmacological remedies; however, it also indicates the distinction between the physiological process of aging and its pathological acceleration caused by other factors.

3. Possible Strategies to Control Aging

The knowledge of the mechanisms underlying the progressive age-related alterations of the organism enables the formulation of three rational strategies to counter aging.

3.1 Telomerase Activation

The methods used for telomerase activation must overcome the fear that telomerase has an oncogenic potential and, therefore, its activation is an oncogenic risk to be avoided [127, 128, 158]. This idea is contradicted by the following findings:

- Old individuals of animal species, such as rainbow trout and lobster, with no age-related decline of survival capacity in the wild (animals with negligible senescence [10]), exhibit the same telomerase activity as young individuals [159, 160]. Because they have constant mortality at any age, their mortality due to cancer does not increase with age;
- Gradual cell senescence is determined by subtelomere inhibition consequent to telomere shortening when telomerase is inactive or partially active (as mentioned in the aforementioned section on gradual cell senescence) and has no likely activity against cancer onset;
- An increasing number of cells in gradual cell senescence or in cell senescence causes the progressive weakening of immune system efficiency [26], which increases vulnerability to cancer and cancer incidence [161];
- Shortened telomeres cause telomere dysfunction and an increasing probability of cancer onset [154, 155, 162];
- Telomerase activation is common in cancer and is a cancer aggravating phenomenon; however, it is subsequent to cancer onset and must not be considered a cause of cancer [26];
- "... short telomeres can actually enhance early steps in tumor formation ... telomerase inhibition could be mutagenic in tumor cells, a lesson that should be held firmly in mind if antitelomerase treatment were being considered as a chemopreventive strategy or were to be used chronically" [163].
- The activation or stimulation of telomerase does not determine an increased risk of cancer and, on the contrary, it reduces this risk compared with untreated age-matched individuals [164].

Despite the feared oncogenic risks caused by telomerase, some in vitro experiments in 1998 demonstrated the positive effects of telomerase activation by stating the following:

- "... two telomerase-negative normal human cell types, retinal pigment epithelial cells, and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced straining for beta-galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already

exceeded their normal lifespan by at least 20 doublings; thus, establishing a causal relationship between telomere shortening and in vitro cellular senescence" [22].

- "... reactivation of telomerase in normal human cells leads to restoration of the length of telomeric DNA and to a highly significant increase in cellular life span. These data provide strong evidence consistent with the telomere hypothesis and indicate that elongation of telomere length by genetic manipulation might render normal human cells virtually immortal" [165].

Similar experiments [166, 167] confirmed these results by indicating that the activation of telomerase maintains cells in a phenotypically youthful state [22]. The following experiments widened these results:

- Fibroblasts aged in vitro and that exhibited "substantial alterations in gene expression" were treated with telomerase activation, and "assessed by incorporation into reconstituted human skin." The reconstituted skin appeared identical to that obtained using young fibroblasts [168].
- In aged mice with blocked telomerase and exhibiting short dysfunctional telomeres and typical degenerative phenotypes, telomerase reactivation extended telomeres and eliminated "degenerative phenotypes across multiple organs, including testes, spleens, and intestines. Notably, somatic telomerase reactivation reversed neurodegeneration with restoration of proliferating Sox2(+) neural progenitors, Dcx(+) newborn neurons, and Olig2(+) oligodendrocyte populations. Consistent with the integral role of subventricular zone neural progenitors in the generation and maintenance of olfactory bulb interneurons, this wave of telomerase-dependent neurogenesis resulted in alleviation of hyposmia and recovery of innate olfactory avoidance responses" [169].
- In normal one- and two-year-old mice, telomerase activation induced by adeno-associated viruses carrying the telomerase reverse transcriptase delayed aging and increased median life span by 24% and 13%, respectively, and "... had remarkable beneficial effects on health and fitness, including insulin sensitivity, osteoporosis, neuromuscular coordination and several molecular biomarkers of aging. Importantly, telomerase-treated mice did not develop more cancer than their control littermates ..." [131].

The activation or reactivation of telomerase, or the stimulation of its activity, can be achieved through two methods.

The first method uses specific drugs. The plant-derived astragalosides stimulate telomerase expression [170, 171]. However, astragalosides are expensive and with limited effectiveness [172]. Other substances capable of stimulating telomerase activity are under consideration (e.g., 08AGTLF, Nutrients 1–4 OA, and MA [173]).

The second method was used in the aforementioned studies [131, 169] and involves the stimulation of telomerase expression by adeno-associated viruses carrying the telomerase reverse transcriptase.

The application of these methods would have the following potential beneficial effects:

- restoration of full functional activity in cells in the gradual cell senescence state;
- reduction in the passage frequency of stem cells to cell senescence state and so to a reduced decline in cell turnover.

The activation of telomerase would not affect the number of senescent cells; moreover, it would not restore the number of stem cells that are present in young individuals.

3.2 Elimination of Senescent Cells

The major features of cell senescence have been explored in Section 2.7. The following findings about the elimination of senescent cells should be added :

- In mice, the transplantation of senescent cells around the knee joints caused alterations similar to osteoarthritis, an age-related pathologic condition [174];
- The elimination of senescent cells through $p16^{Ink4a}$ inactivation reduced and improved age manifestations [122, 123, 175];
- In general, cell senescence is characterized by a single irreversible phase. However, when cell senescence is triggered by certain types of stress, the phenomenon is reversible during the first phase if the stress condition is eliminated or reduced. Moreover, cell senescence is reversible by artificial manipulations in vitro (e.g., by inactivation of both p53 and $p16^{Ink4a}$) [176]. This confirms that cell senescence is a regulated process, i.e., a cellular program [23], but the manipulations performed in vitro cannot be efficiently extrapolated to in vivo conditions. Consequently, the easiest way to achieve useful results is not by bringing senescent cells back to their normal condition but the elimination of senescent cells by using appropriate drugs (defined as senolytics).

Senescent cells have strongly altered functions that should trigger their elimination through apoptosis. However, this does not occur because, as part of the cell senescence program, particular Senescent Cell Anti-apoptotic Pathways (SCAPs) are upregulated, thereby inhibiting the activation of apoptosis. These SCAPs comprise BCL-2/BCL-XL, PI3K/AKT, tyrosine kinase, p53/p21/serpines, and other pathways; these can be mutually related and can constitute broad targets for drugs capable of blocking the aforementioned SCAPs, thereby activating the apoptosis of senescent cells [114].

Dasatinib and quercetin were the first drugs that proved highly effective in eliminating senescent cells [124]. The two drugs act by inhibiting different SCAPs (dasatinib inhibits several tyrosine kinases, while quercetin acts on PI3K and some kinases and serpines, i.e., protease inhibitors), with each of them being more effective for different cell types. Consequently, the combined use of the two drugs, defined as DQ, was proposed and tested on aged mice with positive results (improvement in daily activity, walking speed, muscle strength, and food intake) [124]. DQ also appeared to attenuate various age-associated conditions (e.g., cardiovascular dysfunction) [124].

Subsequently, DQ was further studied in the following studies:

- DQ was used for the treatment of idiopathic pulmonary fibrosis in mice. The treatment improved pulmonary function and physical health; however, lung fibrosis was not modified [177];
- DQ has been studied in clinical trials on patients with chronic kidney disease, diabetes, idiopathic pulmonary fibrosis, and survivors after the transplantation of hematopoietic stem cells [178].

The following natural or synthetic drugs have been proposed as senolytics [179, 180]:

- Navitoclax, the BCL-XL inhibitors (ABT737, A1331852, and A1155463, which target the Bcl 2 family of anti-apoptotic factors), and fisetin, a quercetin-related flavonoid that shows less hematological toxicity than navitoclax [181];
- A senolytic compound (UBX0101) was tested with positive results in transgenic mice for post-traumatic osteoarthritis [182].

- A FOXO4 peptide that alters the interaction of FOXO4 with p53 was tested in mice with doxorubicin-induced chemotoxicity, naturally aged mice, and fast aging XpdTTD/TTD mice. The substance "neutralized doxorubicin-induced chemotoxicity ... restored fitness, fur density, and renal function" [183];
- The small-molecule ABT-737 and siRNAs inhibit the anti-apoptotic proteins BCL-XL and BCL-W and have been tested in transgenic p14(ARF) mice with positive results for epidermis and lung epidermis damage [184].
- Drugs that inhibit the chaperone Heat Shock Protein 90 (HSP90) [125]. The inhibition of HSP90 reduces the resistance of senescent cells to apoptosis [185], an effect applied in cancer treatment [185].

Although several clinical trials are ongoing, senolytics have not been approved for clinical use. Therefore, highly selective senolytic drugs, with the ability to eliminate senescent cells and without any considerable side effects, must be identified. Such drugs should be used to reduce the manifestations of aging and to treat various age-associated diseases.

Senolytic drugs do not affect telomere shortening, the progressive reduction of stem cells, and the consequent decline in cell turnover.

A study proposed that the elimination of senescent cells, after transient positive effects, would cause a strong duplication of stem cells to compensate for the eliminated cells. This would lead to an increase in the passage of duplicated cells to cell senescence, new senescent cells, and an acceleration of clinical disease [186].

The combined use of methods to activate telomerase and drugs to eliminate senescent cells would add up the advantages of the two approaches but would not avoid the decline in cell turnover. Furthermore, in stem cells, the activation of telomerase would elongate telomeres, so reducing the probability of activation of cell senescence in duplicated cells and avoiding the risk of new senescent cells proposed by Fossel [186].

Because the use of senolytics may be associated with possible negative consequences (e.g., the elimination of senescent cells of liver sinusoid endothelial cells appears to cause fibrosis [187]), senomorphics have gained increasing attention as an alternative to senolytics. Senomorphics do not eliminate senescent cells but block or limit the mechanisms and effects of the SASP [188, 189]. However, the distinction between senolyitcs and senomorphics "might be somewhat arbitrary as agents with senomorphic effects in one cell type or context may be senolytic in another and vice versa" [189].

3.3 Restoration of The Number of Stem Cells to That of Young Individuals

Cell turnover decline is the consequence of the progressive reduction in the number of stem cells, which in turn is caused by their random passage to the cell senescence state that does not allow duplication. The probability of this passage is correlated to telomere shortening and is not zeroed even when the shortening is minimal or zero [33].

Telomerase activation lengthens the telomeres and reduces the probability of transition to the cell senescence state; however, it does not restore the number of stem cells to that of a young individual.

Therefore, to counter the alterations of aging, the number of stem cells must be brought back to that of young individuals. As previously mentioned in Section 2.5 (Age-related epigenetic

modifications and their relations with T-sequences), it is possible to transform in vitro adult somatic cells into induced pluripotent stem cells (iPSCs), wherein the epigenetic modifications are practically inexistent. As for embryonic cells [78], MSCs can be transformed into iPSCs [106].

Because the in vivo reprogramming of somatic cells or MSCs into iPSCs does not appear feasible or practical, a possible procedure could be based on the removal of somatic cells, their reprogramming into iPSCs, and their reintroduction into the body.

This procedure, when applied in clinical settings, could integrate telomerase reactivation and the elimination of senescent cells to counter aging.

3.4 Problems Not Addressed by the Aforementioned Strategies

Some characteristics of aging are not resolved by the applications, even if repeated, of the aforementioned three strategies. However, the following methods can be used to improve the results:

- The atrophic syndrome gradually causes irreversible morphological and functional changes. For example, bones weaken to the point that, in some parts, they collapse, and the central nervous system suffers irreversible localized injuries. The three aforementioned strategies cannot reverse these anatomical-functional alterations. However, early and repeated applications of the three strategies should prevent the progression of these alterations.
- Teeth are structures described as perennial. In humans, the teeth are renewed only once, i.e., when they pass from deciduous dentition to permanent dentition. The limit of two dentitions is by no means mandatory for evolution. Among vertebrates, some species have many successive dentitions, such as an elephant having six dentitions [190], and alligators can substitute their teeth up to 50 times [191]. Therefore, the single permanent dentition in humans may appear completely insufficient. However, in natural conditions, the permanent teeth remain in good condition even in old age, whereas in modern conditions, despite the aid of sophisticated dental care, poor condition of teeth is observed in the older population. An extraordinary study of 1939 [192] described how in prehistoric times and in populations living in primitive conditions, caries and other affections of the teeth were practically absent. Therefore, the bad teeth conditions frequent in modern times are the consequence of lifestyles that are profoundly different from those of the prehistoric era.

  To best preserve the teeth, one possible strategy is to adopt lifestyles similar to those of primitive life. This can be combined with the three aforementioned strategies, which would restore to their youthful conditions the parts of the tooth subject to turnover (dental pulp, gums, and mucous membranes of the mouth).

  Moreover, some solutions or palliatives are offered by modern dentistry.

  As a distant prospect in the future, a perfect knowledge of the genetic mechanisms underlying the morphological features of dentition will perhaps make possible specific genetic modifications that will allow multiple dentitions. However, strong ethical obstacles must be overcome.
- The crystalline lens grows continuously, leading to the inability to focus on nearby objects (presbyopia) [193]. The aforementioned procedures would act on the lens epithelium, thereby avoiding the biochemical alterations of the lens body that cause cataracts [194]. However, the three procedures could not stop lens growth and avoid presbyopia. In the future,

the crystalline lens should be replaced with an artificial lens with an elastic capacity to allow accommodation for near vision.

4. Conclusion

Aging is traditionally considered a complex degenerative phenomenon that is difficult and largely useless to counter. The explanation of aging as a physiological, adaptive, and programmed process (therefore, determined and regulated by genes) finds extraordinary support and confirmation in the mechanisms previously exposed and synthetically defined as the subtelomere–telomere theory of aging.

These aging-determining mechanisms exhibit modifiable characteristics. However, for the practical, reliable, and safe control of these modifications, several studies should be conducted.

In the recent past, perhaps still current for many, conceiving the modification or reversal of the aging process was the object of vacuous aspirations or matter for astute charlatans. Today, several researchers consider the modification or even regression of aging as something possible and are willing to invest time, intelligence, and resources.

Furthermore, as a positive collateral action of enormous importance, the strategies indicated to combat aging should also be efficient in treating age-related diseases that are nowadays insufficiently treated or cured with symptomatic therapies and palliatives.

Author Contributions

The author did all the research work of this study.

Competing Interests

No competing interests exist.

References

1. Comfort A. The biology of senescence. London: Livingstone; 1979.
2. Libertini G. An adaptive theory of increasing mortality with increasing chronological age in populations in the wild. J Theor Biol. 1988; 132: 145-162.
3. Nussey DH, Froy H, Lemaitre JF, Gaillard JM, Austad SN. Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. Ageing Res Rev. 2013; 12: 214-225.
4. Oliveira BF, Nogueira-Machado JA, Chaves MM. The role of oxidative stress in the aging process. Sci World J. 2010; 10: 1121-1128.
5. Sanz A, Stefanatos RK. The mitochondrial free radical theory of aging: A critical view. Curr Aging Sci. 2008; 1: 10-21.
6. Weinert BT, Timiras PS. Invited review: Theories of aging. J Appl Physiol. 2003; 95: 1706-1716.
7. Skulachev VP. Aging is a specific biological function rather than the result of a disorder in complex living systems: Biochemical evidence in support of Weismann's hypothesis. Biochemistry (Mosc). 1997; 62: 1191-1195.
8. Libertini G. Classification of phenoptotic phenomena. Biochemistry (Mosc). 2012; 77: 707-715.

9. Pepper JW, Shelton DE, Rashidi A, Dur PM. Are internal, death-promoting mechanisms ever adaptive? J Phylogenet Evol Biol. 2013.
10. Finch CE. Longevity, senescence, and the genome. Chicago: University of Chicago Press; 1990.
11. Libertini G. Non-programmed versus programmed aging paradigm. Curr Aging Sci. 2015; 8: 56-68.
12. Libertini G, Corbi G, Conti V, Shubernetskaya O, Ferrara N. Evolutionary gerontology and geriatrics: Why and how we age. Cham: Springer; 2021.
13. Libertini G, Corbi G, Nicola F. Importance and meaning of TERRA sequences for aging mechanisms. Biochemistry (Mosc). 2020; 85: 1505-1517.
14. Libertini G, Shubernetskaya O, Corbi G, Ferrara N. Is evidence supporting the subtelomere-telomere theory of aging? Biochemistry (Mosc). 2021; 86: 1526-1539.
15. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961; 25: 585-621.
16. Olovnikov AM. [Principle of marginotomy in template synthesis of polynucleotides]. Dokl Akad Nauk SSSR. 1971; 201: 1496-1499.
17. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol. 1973; 41: 181-190.
18. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in tetrahymena extracts. Cell. 1985; 43: 405-413.
19. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990; 345: 458-460.
20. Yu GL, Bradley JD, Attardi LD, Blackburn EH. In vivo alteration of telomere sequences and senescence caused by mutated tetrahymena telomerase RNAs. Nature. 1990; 344: 126-132.
21. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell. 1989; 59: 521-529.
22. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998; 279: 349-352.
23. Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. Int J Biochem Cell Biol. 2005; 37: 961-976.
24. Slijepcevic P, Hande MP. Chinese hamster telomeres are comparable in size to mouse telomeres. Cytogenet Cell Genet. 1999; 85: 196-199.
25. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci U S A. 1995; 92: 4818-4822.
26. Fossel M. Cells, aging and human disease. New York: Oxford University Press; 2004.
27. Seluanov A, Chen Z, Hine C, Sasahara TH, Ribeiro AA, Catania KC, et al. Telomerase activity coevolves with body mass not lifespan. Aging Cell. 2007; 6: 45-52.
28. Gorbunova V, Bozzella MJ, Seluanov A. Rodents for comparative aging studies: From mice to beavers. Age. 2008; 30: 111-119.
29. Kubota C, Yamakuchi H, Todoroki J, Mizoshita K, Tabara N, Barber M, et al. Six cloned calves produced from adult fibroblast cells after long-term culture. Proc Natl Acad Sci U S A. 2000; 97: 990-995.
30. Lanza RP, Cibelli JB, Faber D, Sweeney RW, Henderson B, Nevala W, et al. Cloned cattle can be healthy and normal. Science. 2001; 294: 1893-1894.

31. Herrera E, Samper E, Martín-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. EMBO J. 1999; 18: 2950-2960.
32. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997; 91: 25-34.
33. Blackburn EH. Telomere states and cell fates. Nature. 2000; 408: 53-56.
34. Pontén J, Stein WD, Shall S. A quantitative analysis of the aging of human glial cells in culture. J Cell Physiol. 1983; 117: 342-352.
35. Jones RB, Whitney RG, Smith JR. Intramitotic variation in proliferative potential: Stochastic events in cellular aging. Mech Ageing Dev. 1985; 29: 143-149.
36. Gottschling DE, Aparicio OM, Billington BL, Zakian VA. Position effect at S. cerevisiae telomeres: Reversible repression of Pol II transcription. Cell. 1990; 63: 751-762.
37. Baur JA, Zou Y, Shay JW, Wright WE. Telomere position effect in human cells. Science. 2001; 292: 2075-2077.
38. Baur JA, Wright WE, Shay JW. Analysis of mammalian telomere position effect. Methods Mol Biol. 2004; 287: 121-136.
39. Surace C, Berardinelli F, Masotti A, Roberti MC, Da Sacco L, D'Elia G, et al. Telomere shortening and telomere position effect in mild ring 17 syndrome. Epigenetics Chromatin. 2014; 7: 1.
40. D'Mello NP, Jazwinski SM. Telomere length constancy during aging of Saccharomyces cerevisiae. J Bacteriol. 1991; 173: 6709-6713.
41. Maringele L, Lydall D. Telomerase- and recombination-independent immortalization of budding yeast. Genes Dev. 2004; 18: 2663-2675.
42. Jazwinski SM. The genetics of aging in the yeast *Saccharomyces cerevisiae*. Genetica. 1993; 91: 35-51.
43. Sinclair DA, Guarente L. Extrachromosomal rDNA circles—A cause of aging in yeast. Cell. 1997; 91: 1033-1042.
44. Laun P, Pichova A, Madeo F, Fuchs J, Ellinger A, Kohlwein S, et al. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. Mol Microbiol. 2001; 39: 1166-1173.
45. Lesur I, Campbell JL. The transcriptome of prematurely aging yeast cells is similar to that of telomerase-deficient cells. Mol Biol Cell. 2004; 15: 1297-1312.
46. Herker E, Jungwirth H, Lehmann KA, Maldener C, Fröhlich KU, Wissing S, et al. Chronological aging leads to apoptosis in yeast. J Cell Biol. 2004; 164: 501-507.
47. Büttner S, Eisenberg T, Herker E, Carmona-Gutierrez D, Kroemer G, Madeo F. Why yeast cells can undergo apoptosis: Death in times of peace, love, and war. J Cell Biol. 2006; 175: 521-525.
48. Fabrizio P, Longo VD. Chronological aging-induced apoptosis in yeast. Biochim Biophys Acta. 2008; 1783: 1280-1285.
49. Libertini G. The role of telomere-telomerase system in age-related fitness decline, a tameable process. In: Telomeres: Function, shortening and lengthening. New-York: Nova Science Publishers; 2009.
50. Londoño-Vallejo JA, DerSarkissian H, Cazes L, Thomas G. Differences in telomere length between homologous chromosomes in humans. Nucleic Acids Res. 2001; 29: 3164-3171.

51. Graakjaer J, Bischoff C, Korsholm L, Holstebroe S, Vach W, Bohr VA, et al. The pattern of chromosome-specific variations in telomere length in humans is determined by inherited, telomere-near factors and is maintained throughout life. Mech Aging Dev. 2003; 124: 629-640.
52. Hjelmborg JB, Dalgård C, Möller S, Steenstrup T, Kimura M, Christensen K, et al. The heritability of leucocyte telomere length dynamics. J Med Genet. 2015; 52: 297-302.
53. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol. 1978; 120: 33-53.
54. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. A highly conserved repetitive DNA sequence (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci U S A. 1988; 85: 6622-6626.
55. Blackburn EH. Structure and function of telomeres. Nature. 1991; 350: 569-573.
56. Chen JL. Telomere sequences [Internet]. Telomerase Database; 2020 [cited date 2022 October 6]. Available from: http://telomerase.asu.edu/sequences_telomere.html.
57. Libertini G, Ferrara N. Possible interventions to modify aging. Biochem (Mosc). 2016; 81: 1413-1428.
58. Libertini G. The feasibility and necessity of a revolution in geriatric medicine. OBM Geriatr. 2017; 1: 002.
59. Libertini G, Ferrara N, Rengo G, Corbi G. Elimination of senescent cells: Prospects according to the subtelomere-telomere theory. Biochemistry (Mosc). 2018; 83: 1477-1488.
60. Whittemore K, Vera E, Martínez-Nevado E, Sanpera C, Blasco MA. Telomere shortening rate predicts species life span. Proc Natl Acad Sci U S A. 2019; 116: 15122-15127.
61. Brown WR, MacKinnon PJ, Villasanté A, Spurr N, Buckle VJ, Dobson MJ. Structure and polymorphism of human telomere-associated DNA. Cell. 1990; 63: 119-132.
62. Nergadze SG, Farnung BO, Wischnewski H, Khoriauli L, Vitelli V, Chawla R, et al. CpG-island promoters drive transcription of human telomeres. RNA. 2009; 15: 2186-2194.
63. Azzalin CM, Reichenbach P, Khoriauli L, Giulotto E, Lingner J. Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science. 2007; 318: 798-801.
64. Schoeftner S, Blasco MA. Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat Cell Biol. 2008; 10: 228-236.
65. Vrbsky J, Akimcheva S, Watson JM, Turner TL, Daxinger L, Vyskot B, et al. siRNA-mediated methylation of Arabidopsis telomeres. PLoS Genet. 2010; 6: e1000986.
66. Bah A, Wischnewski H, Shchepachev V, Azzalin CM. The telomeric transcriptome of Schizosaccharomyces pombe. Nucl Acids Res. 2012; 40: 2995-3005.
67. Azzalin CM, Lingner J. Telomeres: The silence is broken. Cell Cycle. 2008; 7: 1161-1165.
68. Diman A, Decottignies A. Genomic origin and nuclear localization of TERRA telomeric repeat-containing RNA: From darkness to dawn. Febs J. 2018; 285: 1389-1398.
69. Porro A, Feuerhahn S, Delafontaine J, Riethman H, Rougemont J, Lingner J. Functional characterization of the TERRA transcriptome at damaged telomeres. Nat Commun. 2014; 5: 5379.
70. Diman A, Boros J, Poulain F, Rodriguez J, Purnelle M, Episkopou H, et al. Nuclear respiratory factor 1 and endurance exercise promote human telomere transcription. Sci Adv. 2016; 2: e1600031.
71. Feuerhahn S, Iglesias N, Panza A, Porro A, Lingner J. TERRA biogenesis, turnover and implications for function. FEBS Lett. 2010; 584: 3812-3818.

72. Chu HP, Cifuentes-Rojas C, Kesner B, Aeby E, Lee HG, Wei C, et al. TERRA RNA antagonizes ATRX and protects telomeres. Cell. 2017; 170: 86-101.
73. Chu HP, Froberg JE, Kesner B, Oh HJ, Ji F, Sadreyev R, et al. PAR-TERRA directs homologous sex chromosome pairing. Nat Struct Mol Biol. 2017; 24: 620-631.
74. Bettin N, Oss Pegorar C, Cusanelli E. The emerging roles of TERRA in telomere maintenance and genome stability. Cells. 2019; 8: 246.
75. Montero JJ, López de Silanes I, Graña O, Blasco MA. Telomeric RNAs are essential to maintain telomeres. Nat Commun. 2016; 7: 12534.
76. Illingworth R, Kerr A, Desousa D, Jørgensen H, Ellis P, Stalker J, et al. A novel CpG island set identifies tissue-specific methylation at developmental gene loci. PLoS Biol. 2008; 6: e22.
77. Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, et al. The NIH roadmap epigenomics mapping consortium. Nat Biotechnol. 2010; 28: 1045-1048.
78. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013; 14: 3156.
79. Lu AT, Fei Z, Haghani A, Robeck TR, Zoller JA, Li CZ, et al. Universal DNA methylation age across mammalian tissues. BioRxiv. 2021. doi: 10.1101/2021.01.18.426733.
80. Rakyan VK, Down TA, Maslau S, Andrew T, Yang TP, Beyan H, et al. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res. 2010; 20: 434-439.
81. Teschendorff AE, Menon U, Gentry-Maharaj A, Ramus SJ, Weisenberger DJ, Shen H, et al. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. Genome Res. 2010; 20: 440-446.
82. Horvath S, Zhang Y, Langfelder P, Kahn R, Boks M, van Eijk K, et al. Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol. 2012; 13: R97.
83. Bell JT, Tsai PC, Yang TP, Pidsley R, Nisbet J, Glass D, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genet. 2012; 8: e1002629.
84. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002; 16: 6-21.
85. Stein R, Razin A, Cedar H. In vitro methylation of the hamster adenine phosphoribosyltransferase gene inhibits its expression in mouse L cells. Proc Natl Acad Sci U S A. 1982; 79: 3418-3422.
86. Hansen RS, Gartler SM. 5-Azacytidine-induced reactivation of the human X chromosome-linked PGK1 gene is associated with a large region of cytosine demethylation in the 5' CpG island. Proc Natl Acad Sci U S A. 1990; 87: 4174-4178.
87. Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, et al. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech Ageing Dev. 2009; 130: 234-239.
88. Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, et al. Aging and environmental exposures alter tissue specific DNA methylation dependent upon CpG island context. PLoS Genet. 2009; 5: e1000602.
89. Pal S, Tyler JK. Epigenetics and aging. Sci Adv. 2016; 2: e1600584.
90. Greer EL, Shi Y. Histone methylation: A dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012; 13: 343-357.
91. Booth LN, Brunet A. The aging epigenome. Mol Cell. 2016; 62: 728-744.

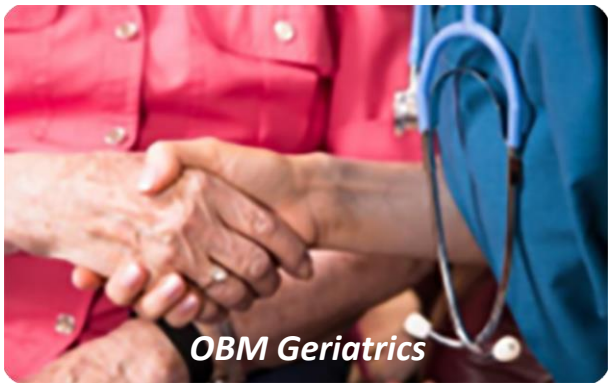
92. Benetti R, García-Cao M, Blasco MA. Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. Nat Genet. 2007; 39: 243-250.
93. Blasco MA. The epigenetic regulation of mammalian telomeres. Nat Rev Genet. 2007; 8: 299-309.
94. Maeda T, Guan JZ, Higuchi Y, Oyama J, Makino N. Aging-related alterations of subtelomeric methylation in sarcoidosis patients. J Gerontol A Biol Sci Med Sci. 2009; 64: 752-760.
95. Buxton JL, Suderman M, Pappas JJ, Borghol N, McArdle W, Blakemore AI, et al. Human leukocyte telomere length is associated with DNA methylation levels in multiple subtelomeric and imprinted loci. Sci Rep. 2014; 4: 4954.
96. Schellenberg A, Lin Q, Schüler H, Koch CM, Joussen S, Denecke B, et al. Replicative senescence of mesenchymal stem cells causes DNA-methylation changes which correlate with repressive histone marks. Aging (Albany NY). 2011; 3: 873-888.
97. Zhou X, Hong Y, Zhang H, Li X. Mesenchymal stem cell senescence and rejuvenation: Current status and challenges. Front Cell Dev Biol. 2020; 8: 364.
98. Moraes F, Góes A. A decade of human genome project conclusion: Scientific diffusion about our genome knowledge. Biochem Mol Biol Educ. 2016; 44: 215-223.
99. Aguado J, d'Adda di Fagagna F, Wolvetang E. Telomere transcription in ageing. Ageing Res Rev. 2020; 62: 101115.
100. Gonzalo S, Kreienkamp R, Askjaer P. Hutchinson-Gilford Progeria Syndrome: A premature aging disease caused by LMNA gene mutations. Ageing Res Rev. 2017; 33: 18-29.
101. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: A continuous and organized process. PLoS One. 2008; 3: e2213.
102. Koch CM. Monitoring of cellular senescence by DNA-methylation at specific CpG sites. Aging Cell. 2012; 11: 366-369.
103. Schellenberg A. Proof of principle: Quality control of therapeutic cell preparations using senescence-associated DNA-methylation changes. BMC Res Notes. 2014; 7: 254.
104. Fernandez-Rebollo E. Senescence-associated metabolomic phenotype in primary and iPSC-derived mesenchymal stromal cells. Stem Cell Rep. 2020; 14: 201-209.
105. Robin JD, Ludlow AT, Batten K, Magdinier F, Stadler G, Wagner KR, et al. Telomere position effect: Regulation of gene expression with progressive telomere shortening over long distances. Genes Dev. 2014; 28: 2464-2476.
106. Spitzhorn LS. Human iPSC-derived MSCs (iMSCs) from aged individuals acquire a rejuvenation signature. Stem Cell Res Ther. 2019; 10: 100.
107. Hynes K. Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. J Dent Res. 2013; 92: 833-839.
108. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007; 130: 223-233.
109. Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, et al. Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell. 2008; 133: 1006-1018.
110. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature. 2003; 426: 194-198.
111. Cristofalo VJ, Pignolo RJ. Replicative senescence of human fibroblast-like cells in culture. Physiol Rev. 1993; 73: 617-638.

112. Kwon SM, Hong SM, Lee YK, Min S, Yoon G. Metabolic features and regulation in cell senescence. BMB Rep. 2019; 52: 5-12.
113. Wang E. Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. Cancer Res. 1995; 55: 2284-2292.
114. Kirkland JL, Tchkonia T. Cellular senescence: A translational perspective. EBioMedicine. 2017; 21: 21-28.
115. Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Curr Biol. 1999; 9: 939-945.
116. van Deursen JM. The role of senescent cells in ageing. Nature. 2014; 509: 439-446.
117. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008; 6: 2853-2868.
118. Rodier F, Coppé JP, Patil CK, Hoeijmakers WA, Muñoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol. 2009; 11: 973-979.
119. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L, et al. Ink4a/Arf expression is a biomarker of aging. J Clin Invest. 2004; 114: 1299-1307.
120. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature. 2016; 530: 184-189.
121. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet. 2004; 36: 744-749.
122. Baker DJ, Perez-Terzic C, Jin F, Pitel KS, Niederländer NJ, Jeganathan K, et al. Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency. Nat Cell Biol. 2008; 10: 825-836.
123. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med. 2016; 22: 78-83.
124. Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. Aging Cell. 2015; 14: 644-658.
125. Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, et al. Identification of HSP90 inhibitors as a novel class of senolytics. Nat Commun. 2017; 8: 422.
126. Campisi J. Cancer and ageing: Rival demons? Nat Rev Cancer. 2003; 3: 339-349.
127. Wright WE, Shay JW. Telomere biology in aging and cancer. J Am Geriatr Soc. 2005; 53: S292-S294.
128. Campisi J. Cancer, aging and cellular senescence. In Vivo. 2000; 14: 183-188.
129. Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, et al. Telomere dysfunction: A potential cancer predisposition factor. J Natl Cancer Inst. 2003; 95: 1211-1128.
130. Ma H, Zhou Z, Wei S, Liu Z, Pooley KA, Dunning AM, et al. Shortened telomere length is associated with increased risk of cancer: A meta-analysis. PLoS ONE. 2011; 6: e20466.
131. Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, et al. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol Med. 2012; 4: 691-704.
132. Biran A, Zada L, Abou Karam P, Vadai E, Roitman L, Ovadya Y, et al. Quantitative identification of senescent cells in aging and disease. Aging Cell. 2017; 16: 661-671.

133. Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov. 2017; 7: 165-176.
134. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell. 2008; 133: 1019-1031.
135. Laun P, Bruschi CV, Dickinson JR, Rinnerthaler M, Heeren G, Schwimbersky R, et al. Yeast mother cell-specific ageing, genetic (in)stability, and the somatic mutation theory of ageing. Nucleic Acids Res. 2007; 35: 7514-7526.
136. Mitteldorf J. Telomere biology: Cancer firewall or aging clock? Biochem (Mosc). 2013; 78: 1054-1060.
137. Stewart JA, Chaiken MF, Wang F, Price CM. Maintaining the end: Roles of telomere proteins in end-protection, telomere replication and length regulation. Mutat Res. 2012; 730: 12-19.
138. Jones M, Bisht K, Savage SA, Nandakumar J, Keegan CE, Maillard I. The shelterin complex and hematopoiesis. J Clin Invest. 2016; 126: 1621-1629.
139. Takai KK, Hooper S, Blackwood S, Gandhi R, de Lange T. In vivo stoichiometry of shelterin components. J Biol Chem. 2010; 285: 1457-1467.
140. Fox SI, Rompolski K. Human physiology, 15th ed. New York: McGraw-Hill Education; 2019.
141. Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972; 26: 239-257.
142. Kaeberlein M, Burtner CR, Kennedy BK. Recent developments in yeast aging. PLoS Genetics. 2007; 3: e84.
143. Benedetti A, Jezequel AM, Orlandi F. A quantitative evaluation of apoptotic bodies in rat liver. Liver. 1988; 8: 172-177.
144. Héraud F, Héraud A, Harmand MF. Apoptosis in normal and osteoarthritic human articular cartilage. Ann Rheum Dis. 2000; 59: 959-965.
145. Richardson BR, Allan DS, Le Y. Greater organ involution in highly proliferative tissues associated with the early onset and acceleration of ageing in humans. Experim Geront. 2014; 55: 80-91.
146. Anversa P, Kajstura J, Leri A, Bolli R. Life and death of cardiac stem cells. Circulation. 2006; 113: 1451-1463.
147. Alberts B, Bray D, Hopkin K, Johnson AD, Lewis J, Raff M, et al. Essential cell biology. 4th ed. New York: Garland Science; 2014.
148. Takubo K, Aida J, Izumiyama-Shimomura N, Ishikawa N, Sawabe M, Kurabayashi R, et al. Changes of telomere length with aging. Geriatr Gerontol Int. 2010; 10: S197-S206.
149. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, et al. Telomere length in the newborn. Pediatr Res. 2002; 52: 377-381.
150. Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. Nat Commun. 2013; 4: 1597.
151. Libertini G, Ferrara N. Aging of perennial cells and organ parts according to the programmed aging paradigm. Age (Dordr). 2016; 38: 35.
152. Libertini G. Programmed aging paradigm and aging of perennial neurons. In: Aging: Exploring a complex phenomenon. Boca Raton: CRC Press; 2018.
153. Libertini G. Programmed aging paradigm: How we get old. Biochem (Mosc). 2014; 79: 1004-1016.
154. DePinho RA. The age of cancer. Nature. 2000; 408: 248-254.

155. Artandi SE. Telomere shortening and cell fates in mouse models of neoplasia. Trends Mol Med. 2002; 8: 44-47.
156. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003; 348: 593-600.
157. Wilson PW, Castelli WP, Kannel WB. Coronary risk prediction in adults (the Framingham Heart Study). Am J Cardiol. 1987; 59: G91-G94.
158. Campisi J. The biology of replicative senescence. Eur J Cancer. 1997; 33: 703-709.
159. Klapper W, Heidorn K, Kühne K, Parwaresch R, Krupp G. Telomerase activity in 'immortal' fish. FEBS Lett. 1998; 434: 409-412.
160. Klapper W, Kühne K, Singh KK, Heidorn K, Parwaresch R, Krupp G. Longevity of lobsters is linked to ubiquitous telomerase expression. FEBS Lett. 1998; 439: 143-146.
161. Rosen P. Aging of the immune system. Med Hypotheses. 1985; 18: 157-161.
162. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. Carcinogenesis. 2010; 31: 9-18.
163. de Lange T, Jacks T. For better or worse? Telomerase inhibition and cancer. Cell. 1999; 98: 273-275.
164. Fossel M, Whittemore K. Telomerase and cancer: A complex relationship. OBM Geriatr. 2021; 5: 156.
165. Vaziri H. Extension of life span in normal human cells by telomerase activation: A revolution in cultural senescence. J Anti-Aging Med. 1998; 1: 125-130.
166. Counter CM, Hahn WC, Wei W, Caddle SD, Beijersbergen RL, Lansdorp PM, et al. Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Proc Natl Acad Sci U S A. 1998; 95: 14723-14728.
167. Vaziri H, Benchimol S. Reconstitution of telomerase activity in normal cells leads to elongation of telomeres and extended replicative life span. Curr Biol. 1998; 8: 279-282.
168. Funk WD, Wang CK, Shelton DN, Harley CB, Pagon GD, Hoeffler WK. Telomerase expression restores dermal integrity to in vitro-aged fibroblasts in a reconstituted skin model. Exp Cell Res. 2000; 258: 270-278.
169. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011; 469: 102-106.
170. Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, et al. A natural product telomerase activator as part of a health maintenance program. Rejuvenation Res. 2011; 14: 45-56.
171. Harley CB, Liu W, Flom PL, Raffaele JM. A natural product telomerase activator as part of a health maintenance program: Metabolic and cardiovascular response. Rejuvenation Res. 2013; 16: 386-395.
172. Fossel M. The telomerase revolution. Dallas: BenBella Books; 2015.
173. Tsoukalas D, Fragkiadaki P, Docea AO, Alegakis AK, Sarandi E, Thanasoula M, et al. Discovery of potent telomerase activators: Unfolding new therapeutic and anti-aging perspectives. Mol Med Rep. 2019; 20: 3701-3708.
174. Xu M, Bradley EW, Weivoda MM, Hwang SM, Pirtskhalava T, Decklever T, et al. Transplanted senescent cells induce an osteoarthritis-like condition in mice. J Gerontol A Biol Sci Med Sci. 2017; 72: 780-785.

175. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011; 479: 232-236.
176. Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, et al. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. Embo J. 2003; 22: 4212-4222.
177. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. 2017; 8: 14532.
178. Fuhrmann-Stroissnigg H, Niedernhofer LJ, Robbins PD. Hsp90 inhibitors as senolytic drugs to extend healthy aging. Cell Cycle. 2018; 17: 1048-1055.
179. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. Nat Med. 2018; 24: 1246-1256.
180. Conti V, Iannaccone T, Filippelli A. Senolytic drugs. In: Encyclopedia of gerontology and population aging. Cham: Springer; 2019.
181. Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell. 2016; 15: 428-435.
182. Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP, et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med. 2017; 23: 775-781.
183. Baar MP, Brandt RMC, Putavet DA, Klein JDD, Derks KWJ, Bourgeois BRM, et al. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell. 2017; 169: 132-147.
184. Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat Commun. 2016; 7: 11190.
185. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer. 2010; 10: 537-549.
186. Fossel M. Cell senescence, telomerase, and senolytic therapy. OBM Geriatr. 2019; 3: 034.
187. Grosse L, Wagner N, Emelyanov A, Molina C, Lacas-Gervais S, Wagner KD, et al. Defined p16(high) senescent cell types are indispensable for mouse healthspan. Cell Metab. 2020; 32: 87-99.e86.
188. Birch J, Gil J. Senescence and the SASP: Many therapeutic avenues. Genes Dev. 2020; 34: 1565-1576.
189. Gasek NS, Kuchel GA, Kirkland JL, Xu M. Strategies for targeting senescent cells in human disease. Nat Aging. 2021; 1: 870-879.
190. Shoshani J. Elephants: Majestic creatures of the wild. New York: Checkmark Books; 2000.
191. Wu P, Wu X, Jiang TX, Elsey RM, Temple BL, Divers SJ, et al. Specialized stem cell niche enables repetitive renewal of alligator teeth. Proc Natl Acad Sci U S A. 2013; 110: E2009-E2018.
192. Elzea CF. Nutrition and physical degeneration: A comparison of primitive and modern diets and their effects. Am J Public Health. 1939: 29: 1358-1359.
193. Petrash JM. Aging and age-related diseases of the ocular lens and vitreous body. Invest Ophthalmol Vis Sci. 2013; 54: ORSF54-ORSF59.
194. Tassin J, Malaise E, Courtois Y. Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. Exp Cell Res. 1979; 123: 388-392.



Enjoy *OBM Geriatrics* by:

1. Submitting a manuscript
2. Joining in volunteer reviewer bank
3. Joining Editorial Board
4. Guest editing a special issue

For more details, please visit:
http://www.lidsen.com/journals/geriatrics