Telomerase and telomeres in aging theory and chronographic aging theory (Review)

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Abstract. The current review focuses on the connection of telomerase and telomeres with aging. In this review, we describe the changes in telomerase and telomere length (TEL) during development, their role in carcinogenesis processes, and the consequences of reduced telomerase activity. More specifically, the connection of TEL in peripheral blood cells with the development of aging-associated diseases is discussed. The review provides systematic data on the role of telomerase in mitochondria, the biology of telomeres in stem cells, as well as the consequences of the forced expression of telomerase (telomerization) in human cells. Additionally, it presents the effects of chronic stress exposure on telomerase activity, the effect of TEL on fertility, and the effect of nutraceutical supplements on TEL. Finally, a comparative review of the chronographic theory of aging, presented by Olovnikov is provided based on currently available scientific research on telomere, telomerase activity, and the nature of aging by multicellular organisms.

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Abbreviations: hTERT, human telomerase reverse transcriptase; TRF1, telomeric repeat binding factor 1; TRF2, telomeric repeat binding factor 2; PB, peripheral blood; HPMCs, human peritoneal mesothelial cells; ALT, alternative lengthening of telomeres; Q-FISH, quantitative fluorescence *in situ* hybridization; SDH, succinate dehydrogenase; FH, fumarate hydratase; IDH1, isocitrate dehydrogenase 1; lncRNAs, long non-coding RNA; tRNA, transfer RNA; rRNA, ribosomal RNA; mRNA, messenger RNA; TERC, telomerase RNA component; PNPase, polynucleotide phosphorylase; PDL, population doublings; TEL, telomere length; PBLs, peripheral blood leukocytes; Flow-FISH, fluorescence *in situ* hybridisation and flow cytometry; WB, whole blood; HSC, hematopoietic stem cells; MSC, mesenchymal stem cells; ATP, adenosine triphosphate; NPC, Niemann-Pick C disease; SMCs, smooth muscle cells; LDL, low-density lipoprotein cholesterol; ROS, reactive oxygen species

Key words: chronomere, stem cells, telomerase, telomere, theory of aging

Contents

1. Introduction

Genetic information is stored in linear DNA molecules - chromosomes in eukaryotic cells (1). McClintock and Möller in 1930 found that complete chromosomes and their fragments behave differently in the cells (2,3). The different properties of chromosomes could be due to the presence of special nucleotide sequences at the chromosomal ends, which are called telomeres (4). Telomeres consist of repeating nucleotide sequences and a set of special proteins that interact with DNA to form a nucleoprotein complex (5). When these repetitive sequences were used for *in situ* hybridization, the following conclusions were made: Human heterochromatin is composed of some heterochromatic pieces, (e.g., that of chromosome) that appear to have more heterogeneous composition than others, while the highly repetitive human DNA fractions are located

primarily at the centromeric and telomeric regions. Moreover, more slowly re-associating repetitious sequences are distributed along the chromatid, with a slight bias towards telomeric regions (6). During each cell division, the 5' end of the newly synthesized strand shortens due to the deletion of terminal RNA primer, leading to incomplete replication of linear DNA molecules. Olovnikov (1971) and Watson (1972) independently formulated the 'problem of insufficient replication' in the 1970s (7,8).

Aging is caused by degenerative diseases, which generates an irreversible accumulation of mutations, inability of cell division, increased susceptibility to morbidity, and ultimately death. Human cells can undergo a limited number of cell divisions and eventually reach an indivisible state called replicative senescence, as telomeres get shorter in each cell division. The link between aging and cancer is a critical aspect of modern research. Unlimited replicative potential, or immortality, is considered the main factor distinguishing cancer cells from normal cells. Numerous studies have shown that tumour cell immortality is associated with the activation of the enzyme telomerase that is responsible for telomere replication and compensates for the telomere shortening that occurs during each cell division. Since the development of methods able to determine telomerase activity, many human tissue samples have been examined for the presence of telomerase activity. Telomerase activity has been recorded in more than 85% of malignant tumours, whereas it was absent in normal tissue cells (9). It is claimed that telomere shortening may be the molecular clock that triggers aging. Studies attempted to verify this hypothesis, by transfecting two telomerase-negative normal human cell types, retinal epithelial cells and foreskin fibroblasts, with vectors encoding the catalytic subunit of human telomerase (hTERT). Unlike the control clones that were telomerase-negative and exhibited telomere shortening and aging, telomerase-positive clones had elongated telomeres and exhibited reduced staining for β-galactosidase, which is an aging biomarker. Importantly, telomerase-positive clones exceeded their normal life expectancy by at least 20 doublings, making it possible to establish a link between telomere shortening and cellular aging *in vitro* (10,11). Moreover, apart from the inhibition of telomerase activity, telomere shortening may be a result of loss of telomere protection and stability. This is caused by the accumulation of unrepaired DNA damage during the cell cycle via destabilization of telomeric protein complexes such as Shelterin complex components i.e. telomeric repeat binding factor 1 and 2 (TRF1 and TRF2) (Fig. 1) (12-15).

2. Telomeres and telomerase: The generation of aging theory

In a historical perspective, Hayflick and Olovnikov greatly contributed to the field of telomeres and telomerase, with the work of Hayflick in 1961, triggering many scientists to try to explain how cells count their divisions (16). He showed that human cells divide only a limited number of times in culture, while at that time the public opinion was dominated by the conviction that T cells are immortal in the hands of a skilled researcher. The article received a tremendous public response and the term 'Hayflick limit' was created and included in the dictionaries.

At first, the telomere functions were unknown, and the sequence of their nucleotides was not known either. In 1958, the DNA polymerase enzyme responsible for DNA replication was discovered by Lehman *et al* (17) and Bessman *et al* (18). DNA polymerase binds to a primer, a short RNA fragment sitting on a DNA strand, that is synthesized by another enzyme in order to initiate replication of telomeres and is subsequently removed. During replication, DNA polymerase moves from the 5'-end to the 3'-end, and as a result, it cannot replicate the entire DNA molecule (there should be a non-copied fragment on one of the ends to which it is attached), the so-called end replication problem. Olovnikov (1971) and Watson (1972) first noticed this in their independent observations (7,8). Later, Olovnikov (1973) published the 'theory of marginotomy' that can be considered a response to the work of Hayflick (1961), explaining how the division counter works (19). In this work it was predicted that in the case of the matrix synthesis of a linear polynucleotide (chromosome replication), the copy should be shorter leading to the limitation of the number of cell divisions observed. Also, the existence of a special enzyme that extends the ends of chromosomes in immortal cells was discussed.

The studies of Greider and Blackburn led the discovery of telomerase in 1984, suggesting that the sequences at the ends of chromosomes may have some special replication mechanism (20). Moreover, Szostak and Blackburn studied the molecular mechanisms of the theory of Möller and McClintock, according to which, telomeres are required for chromosome stabilization (2,3). Importantly in 1988, Harley combined the idea of Olovnikov with the studies of Greider and Blackburn, leading to a breakthrough in the field (21) i.e., observing a shortening of telomeres in human cells overtime (22).

3. Telomerase activity and aging

Telomerase is an enzyme responsible for the replication of the telomeric regions of chromosomal DNA. It is known that shortening of telomeric regions of DNA to critically short length can serve as a signal for the replicative senescence of somatic cells and destabilization of their chromosomes (23). Telomerase activity combined with the telomere length (TEL) reflects the cell's proliferative potential (24). Telomerase activity is mainly present in stem, sex, and tumor cells but there is also evidence of detectable activity in intestinal mucous cells, in lymphocytes of peripheral blood (PB), and thymus (25). In addition, the catalytic subunit of telomerase (hTERT), which is an RNA-dependent DNA polymerase that synthesizes telomeric repeats, as well as the mRNA of this subunit, is also expressed in these cells at a relatively low, but detectable level.

During life, telomeres of PB lymphocytes gradually shorten due to cell division, accumulating with age *in vivo* (25). It has been shown that in many diseases, including immune-associated diseases (rheumatoid arthritis, atopic dermatitis, and bronchial asthma), PB lymphocytes are characterized by shortened telomeres and increased proliferation compared with those in healthy lymphocytes. Numerous studies have shown that telomere shortening may contribute to osteoarthritis and osteoporosis as an epigenetic factor and it is further supported

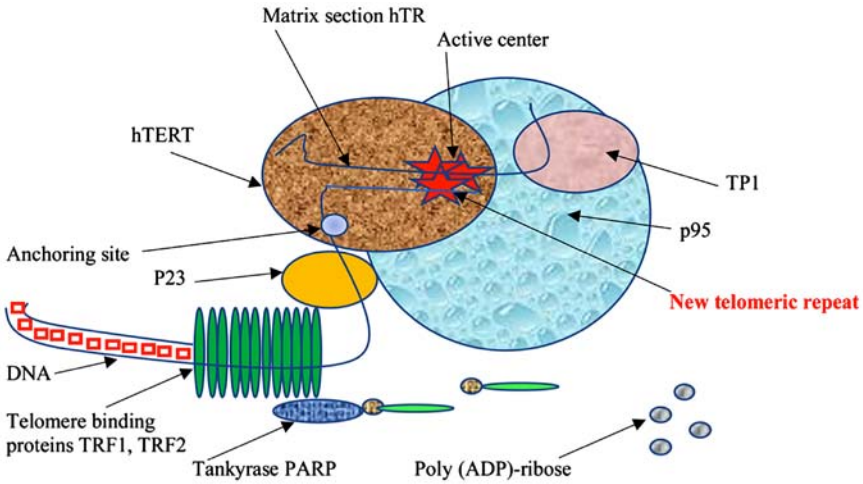

Figure 1. Schematic representation of the interaction of the telomeric complex (comprised of TRF1, TRF2) with a part of telomeric DNA. Protein components include p95, p23 and TP1 proteins, as well as the catalytic subunit hTERT, which performs the synthesis of telomeric repeats. Moreover, tankyrase PARP is depicted to also interact with the telomeric complex. TRF, telomeric repeat binding factor; hTERT, human telomerase reverse transcriptase; PARP, poly (ADP)-ribose polymerase.

that TEL measurement of chondrocytes and PB cells may be an appropriate marker for the evaluation of the progression of these diseases (26-29). Moreover, other factors can influence the rate of telomere shortening in lymphocytes, such as the presence of chronic stress (30) and drug abuse (31). It has been shown that telomerase expression in lymphocytes is strictly controlled during their development, differentiation, and activation (30), and because of stimulation, 'adult' lymphocytes become capable of expressing telomerase at a rather high level. After any re-stimulation, telomerase expression may also increase but its level does not reach the level of response to the primary stimulus (32). Such dynamics may reflect the regulatory mechanisms of clonal expansion of lymphocytes, which is part of the immune response and control mechanism of their proliferation.

Somatic cells are mostly devoid of telomerase activity. It is suggested that telomerase activity can be regulated in human cells by intranuclear transfer of key components. It was shown in HeLa cervical carcinoma cells that hTR and hTERT, during most of the cell cycle, accumulate in intranuclear sites separately from telomeres, whereas during S phase, they are specifically recruited at telomeres. Therefore, the recruitment of telomerase at telomeres is dynamic, reaching a peak in the middle of S phase. Moreover, both hTR and hTERT are associated with nucleoli and Cajal bodies during S phase, participating in telomerase transfer (33). Interestingly, human peritoneal mesothelial cells (HPMCs) appear to have unusually short telomeres (3.5 kbps) despite the presence of active telomerase. These telomeres do not shorten due to aging but due to a decrease in telomerase activity. HPMC aging is associated with mitochondrial dysfunction, leading to increased production of active oxygen species and reduced mitochondrial membrane potential, indicating that premature aging is highly associated with oxidative damage in non-telomeric regions of the genome (34).

Telomerase activity in autoimmune diseases, such as rheumatoid arthritis, lupus erythematosus, atypical dermatitis, psoriasis, multiple sclerosis, and aplastic anaemia is summarized and presented in Table I.

ALT-alternative lengthening of telomeres. In addition to telomerase activation, there is a telomere lengthening mechanism, alternative to telomerase (ALT-alternative lengthening of telomeres). However, this mechanism in mammalian cells is only present in abnormal situations, such as in cancer cells, immortalized cell lines, and murine cell knockout for the telomerase gene, whereas normal human lymphocytes cannot use ALT to maintain their telomeres. A minority of immortal cell lines and tumor cells use ALT to counteract telomere erosion due to cell division (35,36). Observations on the dynamics of the TEL of immortalized cells suggest that this mechanism is based on recombination, resulting in individual telomeres that undergo rapid shortening and rapid elongation (37). Besides, ALT cells can lengthen their telomeres using telomeric sequences from other chromosomes as a template for new DNA synthesis (38,39). Cesare and Reddel (2010) convincingly proved in their research that tumor cells treated with telomerase inhibitors can activate the ALT pathway for telomeric maintenance through recombination (40). This can explain the therapeutic failures and/or a resistance against telomerase inhibition-based anti-cancer therapy. Therefore, developing new therapeutics targeting proteins known to be involved in the latter pathway will be crucial for the targeting of ALT-specific cells (41).

Telomerase is the main mechanism for stabilizing telomeres in lymphocytes and they use it to reduce telomere shortening during cell proliferation (42). Thus, in intact lymphocytes in systemic lupus erythematosus, multiple sclerosis, and atopic dermatitis, telomerase activity is enhanced (43). Moreover, quantitative fluorescence *in situ* hybridization (Q-FISH) on metaphase chromosomes in the PB lymphocytes showed that the distribution of TEL for individual chromosomes differs in normal and pathological conditions. Patients with rheumatoid arthritis had significantly shorter telomeres of chromosome 4p, an observation that could be related to the pathology of the disease, while the activity of the disease is inversely correlated with the length of the telomeres of the 10p chromosome which carries the genes involved in the differentiation and proliferation of T-cells (44). The dynamic activity

Table I. Telomerase activity in autoimmune diseases.

No.	Disease	Telomerase activity in autoimmune disease	(Refs.)
1	Rheumatoid arthritis	The telomerase activity level in synovial infiltrating lymphocytes was significantly correlated with the intensity of synovial lining hyperplasia, microvessel proliferation, lymphocyte infiltration, and percentage of synovial cells positive for proliferating cell nuclear antigen in rheumatoid synovium	Yudoh *et al* (26)
		Activated lymphocytes with high telomerase activity distribute systemically through the circulation and contribute disease activity in connective tissue disease. The stimulatory effect of estrogens on telomerase activity and female predominance in connective tissue disease may contribute to high telomerase activity in these pathologies.	Hiyama and Hiyama (27); Yamanishi *et al* (187); Tarhan *et al* (188)
		Telomerase activity in lymphoid cells of lymphoid organs is higher than in peripheral blood.	Hiyama and Hiyama (27);
		Highest hTERT values were observed in Rheumatoid arthritis group (21.24±28.54). hTERT values were significantly higher than the healthy control group (7.62±4.21) ($P<0.05$).	Tarhan *et al* (188); Yudoh *et al* (26)
		Lack of telomerase activity leads to excessive loss of T cells, which limits the possibility of homeostatic control of naive T-lymphocytes.	Fujii *et al* (28)
		T cells as a result of repeated stimulations correspond to decreasing peaks of mRNA and hTERT induction, compared with the first highest peak.	Valensuela and Effros (141)
2	Systemic lupus erythematosus	High telomerase activity in peripheral blood mononuclear cells.	Katayama and Kohriyama (29)
		Significant correlation between telomerase activity and disease severity. With an exacerbation of the disease, telomerase activity in mononuclear cells of peripheral blood is significantly higher than in the control group.	Klapper *et al* (43); Kurosaka *et al* (189)
		There was no evidence of telomere shortening in lymphocytes and no correlation between telomerase activity and telomere length.	Klapper *et al* (43); Kurosaka *et al* (189)
		The activity of telomerase in the cells is sufficient to compensate for the shortening of telomeres, and thus prevent the accelerated aging of the immune system of patients.	Klapper *et al* (43)
3	Systemic sclerosis	The most interesting finding was very low PBC hTERT levels in the Systemic sclerosis group. If really there is a tendency for lower telomerase activity in Systemic sclerosis, this may be related with the telomere shortening reported by Artlett *et al*. The authors hypothesized that the chromosomal instability observed in Systemic sclerosis patients may result from the loss of long stretches of the telomeric repeats. Telomeric shortening may be expected to stimulate telomerase activation; however, the reverse may also be true. In other words, since the expression of telomerase stabilizes telomere length and allows for continual replication, low telomerase activity may contribute to telomere shortening.	Tarhan *et al* (188); Artlett *et al* (58)
		T lymphocyte homeostasis is significantly impaired.	Hug *et al* (190); Tarhan *et al* (188); Katayama and Kohriyama (29)
		Thymic T cell production was estimated by measuring TCR excision circles (TRECs) as a traceable molecular marker in recent thymic emigrants. an impaired thymic export function and, as a consequence, altered ability to maintain T cell homeostasis and immune tolerance may play an important pathogenic role in relapsing remitting Multiplex Sclerosis.	Hug *et al* (190)
		T cells as a result of repeated stimulations correspond to decreasing peaks of mRNA and hTERT induction, compared with the first highest peak.	Valensuela and Effros (141)

Table I. Continued.

No.	Disease	Telomerase activity in autoimmune disease	(Refs.)
4	Atopic dermatitis and psoriasis	Telomerase activity in the affected areas of the skin is significantly higher than in intact skin and is associated with proliferative activity of the cell.	Jang *et al* (191)
		Lymphocytes that infiltrate the epidermis are not responsible for the level of telomerase, since it does not correlate with the number of these lymphocytes.	Ogoshi *et al* (192); Taylor *et al* (193)
		The level of telomerase activity in skin diseases that are not malignant is much lower than in malignant tumors.	Taylor *et al* (193)
		A high level of telomerase activity in peripheral blood mononuclear cells compared to donors.	Wu *et al* (46)
5		The length of telomeres in blood cells progressively decreases by 216 base pairs in addition to the age loss of 36 base pairs per year.	Brümmendorf *et al* (194); Fogarty *et al* (195)
		The telomere reduction and short cell life can be explained by the presence of mutations associated with this disease in the hTR and hTERT genes.	Ly (196); Young (197); Yamaguchi *et al* (198); Marrone *et al* (199)
		Most patients carrying these mutations are insensitive to immunosuppressive therapy.	Ly (196); Young (197); Yamaguchi *et al* (198); Marrone *et al* (199)
		Among patients with polymorphisms in the TERT gene, the telomere length was within 90% of the values of healthy donors.	Yamaguchi *et al* (198)

of telomerase is correlated with the action of an acute stressor and with two types of reactions to stress-psychological stress and neuroendocrine (cortisol) responses to the stressor (24). Several studies indicate that in most of the above situations (immunopathological diseases, stress, and many others) the telomeres of lymphocytes shorten with age faster than in healthy individuals (45,46).

Role of telomerase in mitochondria. It is known that mitochondrial dysfunctions are found in metabolic disorders, neurodegenerative diseases, and autoimmune diseases (47,48). Mitochondria are important organelles for the production of bioenergy, for biosynthesis and signaling, thus an integral part of cellular adaptation to the environment. Concomitantly, mitochondria are important mediators of carcinogenesis, since this process requires flexibility for the cells to adapt to the environmental changes. Many aspects of mitochondrial biology support transformation, including mitochondrial biogenesis and metabolism, fusion dynamics, regulation of oxidative stress, and cell-signaling. Thus, understanding the mechanisms of mitochondrial function during cancer genesis is critical for future cancer treatment (49). Mutations in mitochondrial genes are common in cancer cells, they do not inactivate mitochondrial energy metabolism but rather alter the mitochondrial bioenergetic and biosynthetic state, including succinate dehydrogenase, fumarate hydratase, and isocitrate dehydrogenase 1, which lead to an increase in the level of succinate, fumarate or R-2-hydroxyglutarate. These metabolic changes can inhibit α-ketoglutarate-dependent dioxygenases, possibly contributing to oncogenesis (50).

Disruption of mitochondrial activity is directly related to neurodegenerative diseases, indicative of the importance of mitochondrial functions in normal cellular physiology. Recent studies focused on mitochondrial function in reactive oxygen generation and its participation in the development of neurodegenerative diseases (51).

As is generally known, the mitochondrial genome is a highly replicated, specialized genetic system that allows individual mitochondria to respond to changes in membrane potential and support oxidative phosphorylation by gene expression (52). Studying this special role, one should not be surprised that the mitochondrial genome is regulated and expressed in a unique way. Human mitochondria contain a compact ring genome of 16,569 bp in length (53). Replication and transcription of mitochondrial DNA (mtDNA) is initiated from the non-coding region, loop D, and is regulated by nuclear-encoded proteins that are post-translationally imported into mitochondria. Mitochondrial RNAs are transcribed as long polycistronic precursor transcripts from 'heavy' and 'light' chains, which are processed according to the 'tRNA punctuation model', whereby 22 disseminated tRNAs are excised to release rRNA and mRNA (54). The released RNAs then undergo maturation, which involves the polyadenylation of 30 ends of mRNA and rRNA (55). Together, this includes a unique genetic system that is able to translate genes encoded by mitochondria into 13 protein subunits of the electron transfer chain. The subtle features of the mitochondrial transcriptome are still poorly understood: Regulation of the number of transcripts, RNA processing and modification sites, and the possible presence of non-coding RNAs. The recent advent of deep sequencing

has provided a global nuclear transcriptome profile, revealing unanticipated transcription complexity, which includes widespread post-transcriptional processing and an abundance of non-coding RNA (56-58). Mercer *et al* (2011) used a similar approach to study mitochondrial transcriptome (59).

Wang *et al* (60) (2010) investigated the import functions of some lncRNAs in mitochondria, showing that one of the RNAs, the RNA component of the telomerase (TERC), is processed in mitochondria and then exported out of mitochondria. Partial disruption of mitochondrial function caused the accumulation of mitochondrial-processed RNA, TERC, in the cytosol. These results provide a potential link between mitochondrial function and telomerase activity. The import of RNA into mitochondria is very important for replication, transcription, and translation of the mitochondrial genome. PNPase is an important regulator of the import of nuclear-encoded RNA into the mitochondrial matrix. It has been shown that the decrease in PNPase impairs mitochondrial RNA processing. Moreover, the increase in imports of RNase P, 5S rRNA, and multidrug resistance proteins RNA is directly dependent on the expression of PNPase. These observations led the identification of PNPase-imported RNA interactions, suggesting a new role of PNPase in providing translocation of RNA to mitochondria (60). In addition, Cheng *et al* (61) (2018) showed that the RNA component of mammalian TERC telomerase is imported into mitochondria, processed into the shorter form of TERC-53, and then exported back to the cytosol. These data collectively reveal the existence of a pathway of mitochondrial RNA transfer and provide a potential mechanism for transferring the functional states of mitochondria to other cellular compartments.

Several recent reports have studied the transport of hTERT to mitochondria, which is most likely controlled by a mitochondrial sequence at the N-terminus of hTERT. It has been shown that the accumulation of endogenous oxidative stress leads to the hTERT nuclear export to the cytosol, reducing the nuclear and total telomerase activity. The addition of antioxidants slows down this process significantly (62). Aging is associated with an increase in the number of intracellular reactive oxygen species (ROS) and a loss of telomerase activity. Haendeler *et al* (62) (2004) investigated whether an age-related increase in the number of ROS can induce nuclear export of TERT and promote endothelial cell aging. Haendeler *et al* found that in parallel with increased ROS formation, the TERT nuclear protein is exported from the nucleus to the cytoplasm and Src kinase is activated. Incubation with N-acetylcysteine reduced the formation of intracellular ROS and prevented mitochondrial DNA damage, as well as low doses of atorvastatin that had a similar effect. Therefore, it has been suggested that antioxidants and statins can delay the onset of replicative aging by counteracting the increase in ROS associated with aging of endothelial cells (62-64).

Telomeric stem cell biology. Most human stem cells are characterized by a rather slow proliferation rate, which was estimated for the hematopoietic stem cells (HSCs), as one division in 1-2 years (65). During the intervals between divisions, the cells are in proliferative rest. However, even with such proliferation rates, it is clear that stem cells have a proliferative potential that is bigger than the Hayflick limit.

The regulation of TEL and telomerase activity is a complex and dynamic process that is tightly linked to cell cycle regulation. HSCs demonstrate telomeric shortening during replicative aging despite expression of low levels of telomerase (66). Granulocytes and naive T cells showed a parallel two-phase decrease in TEL with age, which most likely reflected the accumulated cell divisions in the HSCs that are the common precursors of both types of cells. Memory T cells also first showed a rapid decrease in TEL with age, which continued for several years, resulting in lymphocytes with shorter telomeres that granulocytes in older people. These results indicate a sharp decrease in stem cell turnover in early childhood and confirm that cell divisions in HSCs and T cells lead to telomere shortening (67,68). Telomere shortening is most pronounced during bone marrow resection, as it has been observed that there is an inverse correlation between the number of transplanted mononuclear cells and the degree of telomere shortening. A bone marrow transplant results in a telomere shortening equivalent to a period of 15 years of life (69).

Interestingly, Scheding *et al* (70) (2003) attempted to assess whether repeated stimulation of the hematopoietic system due to regular blood sampling will negatively affect the TEL of PB leukocytes (PBLs). It is known that the TEL of PBLs can be used to evaluate HSCs turnover. A study using fluorescence *in situ* hybridisation and flow cytometry (Flow-FISH), showed that when compared with the corresponding non-donor data, no differences were observed. In addition, neither age-adjusted donor data for TEL for granulocytes nor TEL for lymphocytes correlated with either the total number of years or the total number of whole blood (WB) and/or PLT donations. Long-term donation of WB does not affect TEL, as measured by Flow-FISH, which indicates a significant increase in stem cell turnover (70). Moreover, lymphoid cells are capable of very intensive clonal reproduction with antigenic stimulation. Despite the induction of telomerase, their telomeres are more significantly shortened than in granulocytes. Accordingly, memory cells have significantly shorter telomeres than lymphocytes (71).

There are many unresolved issues in telomeric stem cell biology and the various stem cells of an adult organism vary greatly regarding the role of telomeres and telomerase in maintaining proliferation. These stem cells include HSCs and mesenchymal stem cells (MSCs) from human bone marrow, that are extensively studied. hMSCs are a type of adult stem cells from the bone marrow, which can differentiate into bone, cartilage, adipose, and muscle cells (72). hMSCs show a total absence of telomerase activity, in contrast to HSC and other adult stem cells (73). Ectopic telomerase expression is able to expand their limited replicative ability in tissue culture, maintaining their functional characteristics (74), a feature that can be developed for the application of these cells in tissue engineering (75).

Importantly, stem and cancer cells share several characteristics, including factors that regulate self-renewal in both normal HSCs and leukemic cells (76), leading to the hypothesis of 'cancer stem cells' (77). Cancer stem cells resemble normal stem cells in the sense that when they divide, one of the daughter cells differentiates into a certain type of cell, which eventually ceases to divide, while the other retains its stem cell properties, being able to divide constantly. Cancer stem cells,

which constitute only a small fraction of the total population of tumor cells, are the only tumor cells capable of supporting tumor growth. Therefore, a scientific assumption was made that a complete cure for cancer may require the development of treatments targeting cancer stem cells, assuming that this can be accomplished without destroying the stem cells needed to maintain tissue regeneration, such as bone marrow and intestines (77). In addition, stem cells may be the source of some types of cancer, increasing the risk when used to repair organs, while they can also be of prognostic value as the proportion of stem cells in a tumor can determine how deadly it is.

Some therapeutic strategies for cancer treatment are focused on the destruction of cancer stem cells. However, many potential uses require stem cells to divide to produce enough cells to replace damaged tissue, a fact that could possibly contribute to the accumulation of potentially cancer-causing mutations (77). The origin of stem cells for some types of cancer means that these stem cells have telomerase that does not need to be reactivated, although the enzyme activity may be further increased in the later stages of carcinogenesis (78). On the contrary, the limited activity of telomerase, associated with the final lifespan of adult stem cells, can both contribute to aging and cancer prevention (79,80).

Forced expression of telomerase (telomerization of cells). In many cell types, telomerase activity appears only after the forced expression of the hTERT gene, a process called telomerization. The RNA component of telomerase is expressed in most human cells regardless of telomerase activity; therefore, after the transfection of human cells with the hTERT gene, telomerase activity appears in them, and cells gain the ability to maintain TEL. Such cells are called telomerized. The presence of telomerase activity does not violate the mechanisms of cell growth regulation but it contributes to the stability of the genome, preventing chromosomal rearrangements instead. The sensitivity of telomerized and initial fibroblasts of adult skin to common cytostatics i.e., doxorubicin, cisplatin, etoposide, and dacarbazine was studied. No significant differences in sensitivity were found. These observations directly support the safekeeping of normal mechanisms of homeostasis in telomerized cells and indirectly support the assumption that they can be used in cell therapy (81).

Saretzki *et al* (82) (2004) found very similar molecular events in two unrelated human embryonic stem cell (hESC) lines that regulate the suppression of telomerase activity, increase oxidative stress, and decrease DNA repair activity during spontaneous differentiation. This study characterized resistance to oxidative stress and restoration of DNA breaks in parallel with the regulation of telomerase activity and TEL during differentiation of hESC. Previously, it was reported that murine ESC has a high level of antioxidant protection and effectively repair DNA chain breakage but the level of antioxidant protection and restoration of chain breakdown noticeably decrease during differentiation (82). The data obtained indicated the deacetylation of histones H3 and H4 in the hTERT promoter region. The deacetylation of histone H3 in the hTR promoter was suggested as a candidate mechanism for explaining the suppression of expression of both genes and the loss of telomerase activity during differentiation. Other studies have shown that histone modification is an important means of regulating telomerase gene expression (83,84). The loss of telomerase activity is accompanied by a shortening of telomeres, which is especially fast in the early stages of differentiation. Telomere shortening can be accelerated due to high ROS levels since telomeres are at least partially deficient in repairing oxidative DNA damage (85). It was also shown that the self-renewing potential and longevity of HSCs *in vivo* are largely determined by the ROS levels (86).

Lonergan *et al* (87) (2006) suggested that mitochondrial characteristics may be an additional and reliable way to test stem cell competency. The results indicated that, the perinuclear arrangement of mitochondria in the primate stem cell line, accompanied by a low ATP/cell content and high oxygen consumption, may be a true indicator of competence in stem cell differentiation, and deviations from this profile suggest thaT cells differentiate or possibly age.

Passos *et al* (88) (2007) found in their studies that an increase in mitochondrial density in combination with mitochondrial dysfunction can accelerate aging. This is confirmed by a negative correlation between the density of mitochondria and the maximum life expectancy in interspecific comparisons in mammals. Strengthening mitochondrial biogenesis and, possibly, impaired mitochondrial degradation and segregation, if this occurs as an adaptation to pre-existing mitochondrial dysfunction, it can significantly increase ROS production and, thus, actively contribute to aging. In other words, both ATP and ROS production increase in parallel with the bulk density of mitochondria. In an aging organism, the mitochondrial mass and ROS production increase, while the membrane potential decreases due to the activation of the uncoupling protein (89).

A rather interesting result was obtained after the introduction of the hTERT gene into the cells of a patient suffering from Niemann-Pick C disease (NPC). NPC is a recessive lipidosis that is characterized by excessive accumulation of free cholesterol and glycosphingolipids in the endosomal lysosomal system. Walter *et al* (90) (2003) confirmed that ectopic expression of hTERT in human cells leads to increased regulation of small GTPase Rab9 and its p40 effector. Telomerase immortalization of human cells affects the function of the endosomal lysosomal system and in particular the efflux of cholesterol from this system, independently of the NPC1 protein. Such changes may be necessary to maintain cells in a continuous proliferative state (90,91). In fact, a recent study has provided evidence for a correlation between cholesterol content and cell cycle progression (92). Inhibition of cholesterol biosynthesis with low concentrations of lovastatin blocked cell proliferation. When LDL-cholesterol was added to these cells, cell cycle progression resumed (92). In NPC1 disease, cells are unable to metabolize LDL-derived cholesterol due to its accumulation in the late endosomal lysosomal system. The expression of hTERT in NPC cells leads to the correction of the cell phenotype, including the elimination of the accumulated cholesterol. In particular, in NPC-TERT cells, cholesterol transport from the endosomal lysosomal system to the plasma membrane is restored with a concomitant increase in cholesterol esterification (90).

Transfection of cells with expression vectors containing hTERT supports TEL and provides normal cells with an unlimited life span in culture. It is important that hTERT

prolongs the life span of cultured cells well beyond normal aging without causing a neoplastic transformation (93). Condon *et al* (93) developed a cell line from hTERT-infected human myometrial cells (hTERT-HM). Cells expressing telomerase contained mRNA for α-smooth muscle actin, smoothelin, an oxytocin receptor, and an estrogen receptor α. Myometrial cells immortalized with hTERT retained the differentiation markers that are observed in primary smooth muscle cell (SMC) cultures.

As known, the ectopic expression of hTERT prolongs the lifespan of certain human cells. McKee *et al* (94) (2003) introduced hTERT into human SMCs and found that the resulting cells proliferated well beyond their normal lifespan and, remarkably, retained the characteristics of normal control SMCs. Using these neonatal SMCs, they were able to create reliable human vessels, and then it becomes possible to create coronary arteries for coronary artery bypass grafting. Klinger *et al* (95) (2006) concluded that this tissue engineering model emphasizes the effect of donor age on cellular synthetic function, which apparently does not depend on the increase in hTERT life span.

Chronic stress exposure and telomerase activity. PB mononuclear cells (PBMCs), are most suitable for the study of telomerase activity. Maximow (96) (1909), the founder of the stem cell theory, revealed that PBMCs are stem cells that are common to various blood elements in embryonic development and during the fetal life in mammals. It has been shown that there is an accelerated telomere shortening and reduced telomerase activity in PBMC in various situations. Recent studies have shown changes in TEL and telomerase activity due to psychological and social factors (64). An earlier study showed that telomere shortening and the decrease in the telomerase activity is higher in PBMC of women with high stress levels compared with the control group (24). This study initiated a large number of similar studies, showing that telomeres are shorter in patients with mood disorders (97), in hemodialysis-depended patients (98) and in patients with depressive disorder (99).

To date, the effect of chronic stress on telomerase activity in humans has been described in many studies (68,100-102). This effect includes an increase in the oxidative level, which stimulates hTERT nuclear export to the cytosol and decreases the nuclear and total telomerase activity (62,103,104). It has been observed that long periods of psychological stress can increase vulnerability to the effects of the virus that induces aging of specific T cells (32).

According to Lansdorp (105) (2006), telomere shortening can be due to the influence of psychological stress and social rank, whereas Adams *et al* (106) (2007) contradict the theory that socio-economically deprived people age faster and, therefore, have shorter telomeres than the more affluent part of society. Chronic stress can affect human health through a variety of behavioural aspects and biochemical pathways (107). The shortening of telomeres in cells of the immune system occurs through biochemical reactions (108), while stress can also affect the occurrence of age-related diseases (109). It has been shown that metabolic stress plays an important role in telomere shortening and that the decrease in the level of ω-3 fatty acids in the blood correlates with telomeres and may be a marker of aging (110). Moreover, chronic stress and depression are directly related to high levels of hydroxy-deoxyguanosine and reduced levels of antioxidant enzymes (111,112). In addition, oxidative stress affects telomeres more frequently compared to other areas of genomic DNA and inhibits telomerase activity *in vitro* in various cell types (113,114).

An antioxidant function is associated with various micronutrients, including vitamin C, E, folic acid, and ω-3 fatty acids and, therefore, can positively affect TEL (115). Stress leads to having high levels of pro-inflammatory cytokines, including IL-6 and TNF-α (116,117).

Insulin-like growth factor 1 (IGF-1) exerts a mitogenic effect implicated in cell proliferation. It was first reported that IGF-1 is involved in the modulation of telomerase activity by enhancing the stimulation of telomerase activity caused by phytohemagglutinin (PHA) in cord blood mononuclear cells, which have characteristically low levels of telomerase activity and hTERT expression (118,119).

Fibroblast growth factor 2 (FGF2) has been shown to induce a dose-dependent increase in both neural precursor cell proliferation and telomerase activity in primary cortical cultures (118,120). In contrast, in both neurons and glial cells in primary cultures of E15 mouse embryo cortex, down-regulation of telomerase activity and mTERT gene expression occurred in parallel with cell differentiation (120). In primary endothelial cells, freshly isolated from intact endothelium, telomerase activity is observable during logarithmic growth but not when cells enter quiescence (121). Treatment of quiescent human umbilical vein endothelial cells (HUVECs) with FGF2 reactivates telomerase activity in a time- and dose-dependent manner, in contrast to vascular endothelial growth factor-A (VEGF-A), which has no effect (121). Consistently, FGF2 but not VEGF-A, up-regulates hTERT expression in parallel with transcription factor Sp1 (121).

VEGF has angiogenic activity that can contribute to tumor development by inducing vessel permeability that facilitates tumor cell proliferation and metastasis (122). Although VEGF-A appears not to regulate telomerase activity in human umbilical vein endothelial cells (123), VEGF and lysophosphatidic acid (LPA), both secreted from ovarian cancer cells and known to promote cancer cell growth, have been shown to regulate telomerase activity in non-endothelial cells (118,124). Up-regulation of telomerase activity by VEGF appears to be receptor-dependent, as unsaturated vitamin E, tocotrienol, has been shown to down-regulate telomerase activity through down-regulating the VEGF receptor (125). Vitamin E has also been reported to down-regulate telomerase activity in ovarian cancer cells (118,126).

Genetic factors are also related to telomere shortening. Monoamine oxidase-A and apolipoprotein E (APOE) polymorphisms were statistically significant for TEL only in people with mental and depressive disorders but not in healthy respondents. In addition, sex, the APO-ε2 allele, and the polymorphism of the monoamine oxidase-A have an indirect effect on TEL. Thus, mental and depressive disorder is an additional mediator between monoamine oxidase-A and APOE polymorphisms and TEL (99). Further, studies revealed similar patterns in the European population for the APOE-ε4 gene polymorphism, which is associated with a risk of Alzheimer's

disease. Although telomeres are shortened significantly faster in female carriers of the APOE-ε4 mutant allele, hormone replacement therapy in middle-aged women can change the effect of allele transfer on aging (127).

In order to explain the effect of chronic stress on telomere shortening, Damjanovic *et al* (128) (2007) studied immunological changes in caregivers for relatives with Alzheimer's disease. TEL was significantly shorter in caregivers than in control subjects and their immune response was weaker. Moreover, caregivers had significantly lower T cell proliferation but higher production of immunoregulatory cytokines (TNF-α and IL-10) than control subjects in response to *in vitro* stimulation. These results demonstrate that chronic stress leads to a reduction in TEL and is associated with accelerated aging of T-cells.

However, there is some controversy among different studies on telomere shortening under stress conditions. Effros (129) (2001) proposed an interesting model for the study of PB cells *in vitro*, where lymphocytes from healthy donors, namely men and women aged 25-55 years, were exposed to various concentrations of cortisol, which is a stress-related hormone or dimethyl sulfoxide. He showed that the level of telomerase activity decreases under stress, which explains the decrease in both the number of immune-competent T cells and the length of telomeres in these cells under chronic stress, further accelerating cell aging and deterioration of the immune system (130,131).

Ornish *et al* (132) (2008) investigated for three months the comprehensive lifestyle changes for 30 men with biopsy-diagnosed low-risk prostate cancer, which led to a significant increase in telomerase activity by 10%. Increased telomerase activity was associated with a decrease in the level of low-density lipoprotein cholesterol (LDL) and a decrease in psychological stress. Physical activity appears to protect against metabolic and psychological stress and maintain TEL (133). Moreover, a study on various diets showed that chronic food restriction (regardless of age), body mass index, or smoking might be a risk factor for telomere premature shortening (134). Furthermore, regular sleep, exercise, and a healthy lifestyle contribute to normal TEL and telomerase activity, regardless of the level of the depressive disorder on the Hamilton Depression Rating Scale (135). These data are consistent with the results of another study, which showed that the effectiveness of an antidepressant depends on the initial level of telomerase activity (136). In addition, De Punder *et al* (137) (2018) recently reported that high levels of chronic stress reduce the ability of immune cells to induce telomerase activity by 25% compared to moderate or low levels of chronic stress. Chronic stress is associated with a decrease in mitogen-induced lymphocyte proliferation and mitogen-induced IL-2 production (138), which are activators of signalling pathways that stimulate telomerase activity (139,140). Chronic stress exposure is also associated with a higher level of oxidative stress, which ultimately leads to the accumulation of senescent ($CD28^-$) T-cells (103). This suggests stringent telomerase regulation in human T cells, which may contribute to telomere shortening and ultimate replicative potential and loss of control over certain pathogens (141).

In conclusion, the length of telomeres in PB cells can be an indicator of human health, stress resistance, and cell adaptation. Accordingly, changes in TEL and telomerase activity may reflect the effectiveness of the treatment of age-related diseases. Most of the recent studies on telomeres and age-related diseases are based on the fact that increase in telomerase activity corresponds to either a decrease in telomere shortening (30), or telomere elongation (142).

Effect of TEL on fertility and pregnancy. Vasilopoulos *et al* (143) (2019) investigated the potential relationship between TEL and various factors of female and male infertility. Most female infertility factors have been associated with shorter TEL, with the exception of endometriosis, premature ovarian failure, transparent cell carcinoma, and polycystic ovary syndrome (PCOS). These types of diseases have been associated with a longer TEL, which has shown conflicting results in several studies. On the other hand, male infertility factors were associated with critically shorter TEL (143).

Fragkiadaki *et al* (144) (2016) summarized and critically discussed the evidence linking telomerase activity with pregnancy complications. Maternal age is a determining factor in fertilization success and numerous studies have focused on telomerase activity and its correlation with mammalian fertilization, as well as subsequent splitting processes and preimplantation development. It has been also shown that there is a relationship between telomerase activity and complications during pregnancy.

Additionally, the effect of psychosocial stress on pregnancy is an important risk factor for the early onset of common age-related diseases (145). Human studies have demonstrated a link between chronic or excessive psychosocial stress and telomere biology (146). It was shown that stress-related changes in telomeres might be a possible mechanism linking psychosocial stress with age-related diseases (147). Indeed, the accelerated shortening of telomeres reflects stress-induced oxidative cell damage and accelerated aging (97). Therefore, Lazarides *et al* (148) (2019) examined the hypothesis that the maternal pro-inflammatory state during pregnancy, which is a balance between TNF-α, the main pro-inflammatory cytokine, and IL-10, the main anti-inflammatory cytokine, are directly related to the TEL of the leukocytes (LTL) of the newborn at birth. The higher average ratio of TNF-α/IL-10 during pregnancy was significantly associated with shorter TEL of the newborn after adjusting for the mother's age, body mass index before pregnancy and sex of the child.

Effect of nutraceutical supplements on TEL. The Dietary Inflammatory Index (DII) was developed by Shivappa *et al* (2014) to measure the inflammatory potential of diet and it can be used in diverse populations to predict levels of inflammatory markers, including C-reactive protein (CRP) and interleukin-6 (149-151). Shivappa *et al* (152) (2017) examined whether inflammatory potential of diet, as measured by the Dietary Inflammatory Index (DII) has an impact on telomere shortening in the National Health and Nutrition Examination Survey (NHANES). Validation of the DII with C-reactive protein (CRP) was also carried out, showing that there was an association between DII and leukocyte TEL in these NHANES data. The impact of specific fatty acids on inflammation can be crucial to the effect of dietary fats on human health, for example polyunsaturated fatty acids,

mainly ω-3 fatty acids, have anti-inflammatory and immunomodulating properties. Research studies have confirmed that excessive amounts of ω-6 fatty acids leading to a high ω-6: ω-3 fatty acid ratio can promote the pathogenesis of chronic diseases (153). Interestingly, a Mediterranean diet supplemented with two fatty fish meals per week, rich in ω-3 fatty acids, has been shown to have an anti-inflammatory effect reducing airway inflammation in childhood asthma that is a chronic disease (154). Therefore, it is likely that blood polyunsaturated fatty acid levels are involved in preventing telomere shortening over time (155). In agreement with this, recent studies revealed that the administration of nutraceutical supplements to healthy individuals is implicated in TEL maintenance (156). Participants were selected from healthy outpatients and divided into an intervention group that received nutraceutical supplements and a control group. The length of individual telomeres was estimated in metaphase leukocytes isolated from PB using Q-FISH analysis. The median length of the telomeres was significantly increased ($P<0.05$) in the intervention group compared with the control group. The beneficial effect of supplements on the length of short telomeres was significant ($P<0.05$) after correction for age and sex, suggesting that nutraceutical supplements can directly affect the maintenance of TEL.

4. Chronographic theory of aging: Neural chronograph

As mentioned, Olovnikov (1973) described the 'problem of insufficient replication' in the 1970s and predicted that in the case of the matrix synthesis of a linear polynucleotide (chromosome replication), the copy should be shorter and the limitation of the number of cell divisions is associated with this fact (19). This led him to construct a new theory of aging (160,161). It has been proposed that if development and aging are programmed, then some 'watch' mechanism that controls aging is required (67,162-165). Biorhythms are often called clocks, and genes involved in rhythms, usually circadian rhythms, are referred to as clock genes. But is there a real time-keeping mechanism in the body that would organize, register, and track physiological changes according to the age of the body? Olovnikov bases his theory on the idea of the controlled loss of neurons in the brain of hypothetical organelles, called chronomeres, which represent small DNA molecules that were amplified from chromosomal DNA segments (162,163).

Chronographic theory of development and aging. A biochronograph to describe the development, aging, and longevity of a certain species, should consider that some genes have probably changed their expression in different species and in different age groups. In *C. elegans*, for instance, a relatively small number of genes show significant changes in transcript levels as they age (<1% of the genome) (166). Comparison of the results of the *C. elegans* shows sets of overlapping genes that are highly conserved throughout evolution and appear to be genes that actually control aging and lifespan (167,168). Herndon *et al* (169) (2002) found convincing evidence that stochastic as well as genetic factors are important in aging *C. elegans,* with extensive variability between cells of the same type within individuals. Moreover, the timing of gene expression of adult *Drosophila* is regulated by independent mechanisms related to temperature and metabolic rate. Studies of relative temporary scaling at long-living mutants of *C. elegans* showed that the life expectancy is controlled by some physiological hours different from chronological time (170).

Olovnikov (171) (2007) claims that the lunar cycle plays a role in aging of living organisms. He suggested that pineal cells called pinealotcytes regularly change the endocrine secretory activity, responding to periodic changes of the gravitational field of the moon. The theory is based on the idea of a controlled loss by a neuron in the brain of chronomeres. The regular process of loss of chronomeres in neurons is controlled by the pineal gland and activated at least once a lunar month. Hormonal signals can be generated by the pineal gland and depend on the gravity of the periodic shift of the mineralized deposits located at the intra-pinealotcyte of neighbour cellular structures. The calcific large particles that are in extracellular spaces of a pineal gland can also contribute to the hormonal secretion.

This pineal secretion activates the hypothalamus, enhancing the hormonal response, which allows a sharp increase in the concentration of several hormones, the so-called T-signal. The T-signal is a 'hormonal intracerebral explosion' that happens in humans every month and is a kind of hormonal combination, which can enhance transcription in brain cells. Under certain conditions, the chronomeres in response to a T-signal can lose each neuron performing temporary functions, and as a result, the neuron changes the activity. As mentioned above, chronomeres are hypothetical mini-organelles that are present in the neurons performing temporary functions, including counting time and participating in the control of the biological age of an organism. Chronomere is a perichromosomal copy of the chromosomal original, a rather small two-chained DNA molecule, which is protected by proteins. Thus, each chronomere is on a surface of a chromosome and is levelled along this segment of chromosomal DNA, which serves as the original for this chronomere. Chronomere DNA contains various regulatory sequences (171).

Adjusting the sensitivity of endocrine gravity sensors in different species of vertebrates may be one of the key factors responsible for the rate of embryonic development in each species of vertebrate. Gradual suppression of inhibitors is very important for embryonic brain development (172,173). Changes in functional inhibitory interactions are necessary for the maturation of the prefrontal cortex when the cerebral cortex is connected (174). Epigenetic suppression of key inhibitory loci may play a fundamental role in the initiation of puberty (175). When the maturation of the body is complete, clusters of neurons remain in the brain, which are essentially the endpoint of the time relay, while the temporary neurons of these clusters contain only terminal chronomeres, which gradually decrease. The greater the reserves of such neurons, the longer the lifetime is, considering that all other things are equal, which could be the reason why life expectancy is positively correlated with the cephalization index (176).

Age-related changes in the human brain as a possible outcome of the chronomere's function. Dedifferentiation of brain cells is used as a means of development and loss of control and dedifferentiation of neurons is found in several pathologies (177). However, changes in the brain must also occur

during normal development. Dedifferentiation or decreased treatment specificity is a robust characteristic of cognitive aging. Voss *et al* proved that neural dedifferentiation was not ubiquitous in different categories of stimuli, while nervous dedifferentiation was relatively stable according to the age of the elderly (178,179). Moreover, an age-related decline in specialization has also been shown for the cognitive function of the frontal lobes of the hemispheres (180). Dreher *et al* examined the relationship between dopamine synthesis in the midbrain and the prefrontal ligament with reward. It was found that healthy aging causes functional changes in the reward system, and reveals age-related changes in the relationship between dopamine synthesis in the midbrain and prefrontal activity. These results gave insight into the interaction between dopamine function of the midbrain and the reward system in young people and the elderly, and also determined the changes that accompany aging (181). Furthermore, it has been shown that the contraction of the hippocampus, inferior temporal cortex and prefrontal white matter are increased with age, while shrinkage in the hippocampus and cerebellum accelerates (182,183). The weakening of the functional correlations of individual parts of the brain spreads with age from the forehead to the back of the head (184).

The decrease in the volume of a brain becomes noticeable approximately from 30 years of age (185). Total brain volume for mentally healthy people is steadily declining by 0.22% per year between 20 and 80 years, and this decrease is accelerated in older age (186). The brain in some patients has vital deposits of amyloid protein indicative of the preclinical phase of Alzheimer's disease: Their brain volume is reduced by 2.5% compared with the brain of healthy volunteers. All of the listed age-related changes, including a decrease in brain volume, are considered a result of aging caused by various factors, among which is the accumulation of damage caused by free radicals. However, qualitative changes in the brain can be interpreted in a completely different way, meaning that all these changes in the brain are not the result of errors, but the product of genetic programming.

5. Conclusions and future research directions

As discussed in this review, telomerase activity is regulated in response to various biological factors and systems, including stress hormones, oxidative stress, inflammatory mediators, and circadian rhythm. Therefore, future studies of telomerase activity in PB mononuclear cells should include measurements of biomarkers that participate in these processes, which will significantly contribute to enhancing our knowledge in the field. Moreover, it is necessary to further study the role of telomerase in the defence mechanism of mitochondria against oxidative stress to gain a broader understanding of the role of telomerase in human health and the risk of stress-related diseases.

A decrease in telomerase activity may explain why telomeres shorten faster under pathological conditions. The study and characterization of individual differences in the ability of the telomere biology system to respond to a challenge, in addition to indicators of basal telomerase activity and TEL, is an interesting non-standard approach for future studies of telomere biology and aging.

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Authors' contributions

All the authors contributed in conceiving and designing the study. MPR, AMZ and KSG searched the literature for inclusion in the study and wrote the manuscript. MT, ES, and PF checked and reviewed the manuscript. KN, DT, DAS, and AT gave advice and critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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Competing interests

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