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Telomeres: Chromosome End Protective-Complexes and Its Association with Chronic Diseases

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Abstract

Telomeres are specialized nucleoproteins present at the end of linear eukaryotic chromosomes. They consist of a double-stranded DNA sequence and a single strand DNA protrusion (free 3'OH end). Telomeres are assembled into a three-dimensional structure in association with the shelterin complex. Telomeres play a central role in chromosomal stability and proliferative history of the cells. Shortening of telomeres is considered an important marker of cellular aging shortening in a physiological way in each round of cell replication in somatic cells. More impressive, telomeres' attrition is highly susceptible to deterioration related to DNA damage accumulation during the aging process. Clinical evidence suggests that telomeres' shortening contributes to the establishment and progression of the aging phenotype in some inflammatory chronic disorders. In other cases, experimental evidences suggest that aging is accompanied with an abrupt shortening in the context of such diseases, proposing that the length of telomeres may be an important biological marker for progression of various pathologies.

Keywords: Aging; Telomeres; Telomerase; Shelterin complex; Chronic diseases

Abbreviations

AA: Aplastic Anemia; ATM: Ataxia Telangiectasia Mutated; ATR: Ataxia Telangiectasia Rad3-related; B/F/B: Breakage/Fusion/Bridge cycles; BD: Bipolar Disorder; CCL11: C-C Motif Chemokine Ligand 11; CDKN1A: Cyclin Dependent Kinase Inhibitor 1A; COPD: Chronic Obstructive Pulmonary Disease; CTC1: CST Telomere Replication Complex Component 1; DC: Dyskeratosis Congenita; DDR: DNA Damage Response; DKC1: Dyskerin; DNA: Deoxyribonucleic Acid; DSB: Double Strand Break; dsDNA: double-stranded DNA; Exo1: Exonuclease 1; HDR: Homology-Directed Repair; HHS: Hoyeraal-Hreidarsson Syndrome; IPF: Idiopathic Pulmonary Fibrosis; kb: kilobase; NHEJ: Non-Homologous End Joining; PBMC: Peripheral Blood Mononuclear Cells; POT1: Protection of Telomeres 1; RAP1: Ras-Related Protein 1; RNA: Ribonucleic Acid; SSB: Single Strand Break; ssDNA: single-stranded DNA; STN1: STN1 subunit of CST complex; SZ: Schizophrenia; TCAB1: Telomerase Cajal Body Protein 1; TEN1: TEN1 subunit of CST complex; TERC: Telomerase RNA Component; TERT: Telomerase Reverse Transcriptase; TIN2: TRF1 Interacting Nuclear Factor 2; TP53: Tumor Protein P53; TPP1: Tripeptidyl Peptidase 1; TRF1: Telomeric Repeat Binding Factor 1; TRF2: Telomeric Repeat Binding Factor 2

History of the Telomeres

The possibility of the presence of specialized structures at the end of the chromosomes emerged in 1938 when Herman Muller observed that X-rays could cause breaks in the chromosomes of the fruit fly *Drosophila melanogaster* and that the fusions did not occur at the ends of the chromosomes [1]. Simultaneously, in an independent study, Barbara McClintock observed a similar process after induction of chromosomal breaks in corn species [2]. The ability to avoid fusion of their ends led to the conclusion that the chromosomes were protected by some structure, called by Muller telomeres.

Years later, Leonard Hayflick observed *in vitro* that mouse fibroblasts had limited proliferative potential and accumulated aging-related characteristics, suggesting that these cells were not able to

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divide indefinitely. The critical moment of cell cycle arrest was called the "Hayflick limit" [3]. A few years later, studies on the properties of the DNA semi-conservative replication process [4,5] revealed particularities that corroborated Hayflick's theory of senescence.

In 1978, Elizabeth Blackburn and Joe Gall observed the repetition of the 5'-TTGGGG-3' hexamer at the DNA molecules on chromosome ends through sequencing the macro nuclear genome of *Tetrahymena* [6]. In 1982, Jack Szostak confirmed the conservation of telomere function throughout the evolution of the species, demonstrating that the linearization of yeast circular DNA could be stabilized after recombination with the telomere sequence of the protozoan *Tetrahymena* [7]. Since that, several studies emerged with the intention to investigate the function of telomeres biology and its association with diseases.

Structure and Function

Telomeres consist of a non-coding nucleotide sequence, composed by a double-stranded DNA sequence and a single strand DNA protrusion composed of 150-200 nucleotides (Figure 1), stabilized by proteins that forms an intricate structure, preventing telomeres been recognized as a single (SSB) or double DNA Strand Breaks (DSB). The single-stranded DNA (ssDNA) overhang consists of guanine-rich repeats at the 3'OH end, called the G-strand. The complementary 5' strand is rich in cytosine and named as C-strand [8].

The molecular analysis demonstrates that telomeric DNA forms a stable structure, in which the 3OH overhang is rearranged, inserting into the double-stranded DNA (dsDNA), forming a lariat structure, called the T-loop [9]. The final, single-stranded portion of the T-loop is protected by the ssDNA, disrupting base pairing between the double helix, forming a portion of triple-stranded DNA, called the D-loop (Figure 2) [10]. This specialized structure prevents the 3OH overhang from being recognized as an SSB, thus inhibiting the activation of the DNA Damage Response (DDR)-signaling machinery induced by Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia Rad3-related (ATR), key pathways that promote end-to-end fusion by Homology-Directed Repair (HDR) and Non-Homologous End Joining (NHEJ) [11].

The number of replicates of the telomeric sequence widely diverse between species, but its sequence is highly conserved in eukaryotic [12]. In mammals, the nucleotide sequence consists of the TTAGGG tandemly repeated hexamer, but the number of replicates is also variable between tissues and cells within the same organism. In humans, the size of telomeres can vary between 10-15 kilobases (kb) in early life, and even in some mouse strains, which can be as long as 40-80kb [13].

Thus, telomeres play an important role in protecting the genome against nucleolytic degradation, spontaneous recombination, repair events that result in chromosomal fusion and preventing SSB/DSB [14]. Moreover, telomeres are essential regulators of chromosomal positioning and cellular replicative capacity [15], conferring chromosomal stability and maintenance of genomic homeostasis.

The telomerase enzyme

In the early 1970s, Alexey Olovnikov hypothesized that a particular enzyme might be able to compensate for the loss of nucleotides resulting from the end replication problem [5]. In this sense, Telomerase was first described in 1987 by Carol Greider and

Elizabeth Blackburn [16]. Telomerase is a reverse transcriptase enzyme responsible for the *de novo* synthesis of the telomeric DNA, being the main physiological mechanism by which mammalian cells extend their telomeres. In humans, telomerase adds the TTAGGG hexamer to the 3'OH overhang at the end of linear chromosomes [17].

The telomerase catalytic core is a ribonucleoprotein complex composed of a polypeptide subunit termed Telomerase Reverse Transcriptase (TERT) and a Telomerase RNA Component (TERC) belonging to the non-coding RNA family [18] (Figure 3). Telomerase activity counteracts the natural shortening of telomeres associated with cell replication and DNA degradation events [19]. Its up regulation or mutation is a strategy described in major types of cancers for unlimited replicative capacity [20]. Biogenesis and maturation of the telomerase complex, formed by its two major TERT and TERC subunits, occurs through associations with specific proteins and domains in the Cajal body [18], a highly conserved organelle specialized in the maturation of ribonucleoproteins [13]. After assembly of the functional holoenzyme, additional proteins, such as TPP1, TIN2 regulate the recruitment of TERT to the 3'OH free end of the G-rich leading strand, a process facilitated by the Telomerase Cajal Body Protein 1 (TCAB1) [21-24] and Dyskerin (DKC1), that interacts with specific non-coding RNA domains, conferring stability to the structure [25]. The telomerase complex is further associated with components that assure its *in vivo* activity. In this regard, the shelterin proteins are interconnected ss and dsDNA, assisting with the holoenzyme recruitment and activity [26,27].

The shelterin complex

The ends of the chromosomes are protected by a complex consisting of six DNA binding proteins, called the shelterin complex, which in turn associates with other proteins, and complexes (Figure 2), conferring structure stabilization and controlling the length of the telomeric DNA [28].

Telomeric Repeat Binding Factor 1 (TRF1) and 2 (TRF2) are independent proteins that bind to DNA in association with the protein of Protection of Telomeres 1 (POT1) and interact with the telomeric sequence, forming dimers or multimers [29]. The TRF1, TRF2, and POT1 proteins interact through a bridge formed by two other proteins, the Tripeptidyl Peptidase 1 (TPP1) protein and the TRF1 Interacting Nuclear Factor 2 (TIN2), which do not bind directly to the DNA strand [12]. The sixth component of the shelterin complex, the Ras-Related Protein Rap1 (RAP1), is the evolutionarily most conserved protein in the complex, interacting exclusively with TRF2 [26].

The formation of TRF1 protein homodimers with double-stranded telomeric DNA is presumed to monitor sequence length, whereas homodimers formed by the TRF2 protein are able to stabilize T-turn formation by protecting the 3OH ribbon protrusion from the G-strand [30]. TRF1 also may act as an inhibitor of the telomerase enzyme, preventing the elongation of the telomeric DNA [29].

The TIN2 protein binds to the complex formed by TRF1 and TRF2 in association with double-stranded DNA, bridging with POT1 through TPP1. POT1 is the only protein in the shelterin complex that binds to the 3'OH protrusion of the G-strand. Thus, the six-component polypeptides of the complex form two compartments with the telomeric structure. In one, the proteins are bound only to double-stranded DNA, while in the other; the proteins are bound in both the double-stranded telomeric region and the 3OH protrusion

of the G-stranded ribbon [12].

The TRF1 protein has the function of controlling the size of telomeres through the maintenance of telomeric region replication [31,32]. Studies have demonstrated that TRF1 assists in the maintenance and direct regulation of telomere length by interacting with TIN2, TPP1 and POT1 proteins [33], and its depletion is related to increased DNA damage response, accelerating the aging phenotype [34]. The TRF2 protein stabilizes the T-loop in the telomeres by modifying DNA topology induced by positive super coiled conformational structures that protect the 3'OH overhang from NHEJ, directly suppressing ATM-dependent DDR signaling [35-37].

In addition, the interaction of the RAP1 and POT1 proteins is responsible for preventing events of homologous recombination [30]. In humans, the association of RAP1 with telomeres is stabilized by the TRF2 protein [38], with the auxiliary function of preventing homologous recombination and preventing telomere elongation independent of telomerase action. The interaction of TPP1 and POT1 also plays a suppressive role in homologous recombination [26], and in the inhibition of pathways that activate DDR [29]. In addition, other components of the shelterin complex may limit the bioavailability of POT1 in the telomeric structure, depending on the amount of the dimer formed by the binding between TPP1 and TIN2 [26]. Overexpression of TIN2 inhibits telomeres' elongation in human cells of the immune system, whereas inhibition of the gene encoding that protein results in uncontrolled elongation of the telomeric sequence. In addition, it has been suggested that the binding of TIN2 to TRF1 induces changes in the formation of TRF1, favoring the structure of telomeres, making it inaccessible to the action of telomerase [39].

Mutations in the genes encoding the proteins of the shelterin complex can cause recombination of telomeric DNA strands, leading to telomere dysfunction, cell cycle arrest, and apoptosis [40]. Evidences have been suggested that the shelterin binding requires a minimum telomeric DNA length in order to maintain regulatory functions and telomere protection [41]. Thus, the integrity and function of telomeres are directly associated with the complex, as well as the bioavailability of each component and its interactions.

Telomeres' replication

Linear chromosomes from eukaryotic organisms have protrusions at the 3'OH ends [42]. The leading strand is continuously extended in a 5'-3' direction towards the replication fork opening. However, the lagging strand is discontinuously synthesized by multiple small DNA fragments, called Okazaki fragments [43] and need additional exonuclease and DNA ligase1 activities to stabilize the newly formed strand [44,45]. In this sense, due to the inability of the DNA polymerase to completely replicate the discontinuous strand at the very last distal Okazaki fragment, the DNA replication machinery generates a 3'OH overhang at the end of the process [46]. This natural cellular process, called end replication problem, was first described by Jim Watson, who noticed the progressive loss of nucleotides at the 3'OH end of the chromosomes, within each cell division [4]. Physiologically, the number of nucleotides that are removed from each cell division is not constant and depends on several factors, such as the position and size of the nucleotide sequence of the last RNA primer and the length of the DNA sequence [46].

In addition to the end replication problem, mammalian telomeres

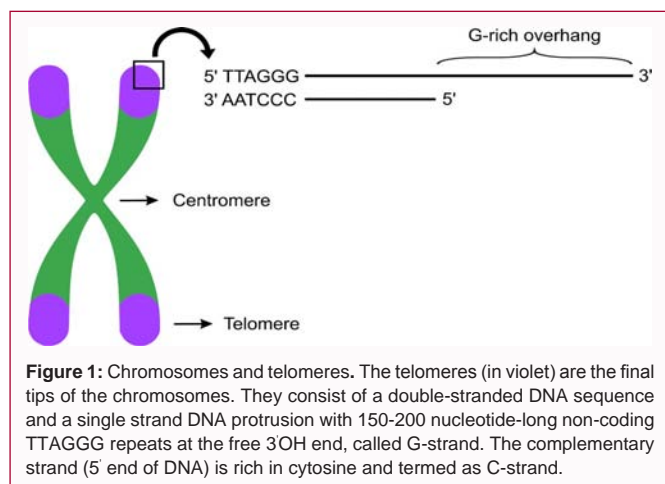
are also shortened through a 3'OH nucleases- dependent resections accomplishment mechanism. After DNA replication process, TRF2 recruits both nucleases Apollo and Exo1 in order to resect the leading and lagging ends on telomere DNA, and Exo1 to generate a functional 3'OH overhang as a multi-step, shelterin-controlled process [47]. Afterward, POT1 directs the overhang fill-in synthesis in late S phase by the recruiting Pol- α through a three-protein complex named CST, composed by CTC1, STN1 and TEN1 proteins [48].

Telomeres as markers of biological age

As in mammals, telomerase activity is the major mechanism to avoid excessive telomeres' attrition; its expression and regulation are tightly modulated [49-53]. In this sense, although TERC subunit is ubiquitously expressed in mammal tissues, TERT subunit is only expressed in most embryonic stem cell compartments, germ-line and cancer cells, and necessary for counteracting excessive telomeres' attrition [54-57]. Some embryonic stem cells, such as hematopoietic precursors, are characterized by reduced telomerase activity, allowing partial compensation of telomeres shortening. Somatic cells, such as differentiated circulating immune system cells, are generally characterized by the absence of telomerase activity by suppressing TERT expression, implying in limited proliferative capacity [58].

Thus, the natural process of telomere shortening may represent the pace or rhythm of the aging process and can be compared to a mitotic clock, reflecting the proliferative history of the cells [14]. This definition suggests that the length of telomeres and their shortening rate could be considered a biological age marker, both at the cellular and systemic status, representing an objective measure of events accumulated over organism's lifespan. In this regard, the accelerated attrition of the telomeric sequence could be associated with a potential biomarker related to the early onset of aging [59].

Telomeres homeostasis is species-dependent and tissue-specific. Its shortening rate varies with age and cell type [60]. During embryonic development of vertebrates, telomere length remains constant in most tissues by active telomerase. However, after birth, somatic cells undergo progressive telomeres shortening due to telomerase inactivation through regulation [61]. In this way, variations in the rate of telomeres attrition are directly correlated with its proliferative capacity. For example, high self-renewal tissues, such as intestinal mucosal and Peripheral Blood Mononuclear Cells (PBMC), are associated with accelerated shortening of their telomeres. On the other hand, tissues with lower turnover, such as neurons and myocytes, are characterized by attenuated telomeres' attrition [62]. As mentioned before, the successive loss of nucleotides at each cell replication cycle is a physiological process. However, as we have seen, when telomeres reach a critical length, it may induce DDR signaling, resulting in cell cycle arrest and the transcription of genes that activate cellular senescence pathways [63]. Indeed, cell cycle arrest is stabilized by signals that activate the TP53/CDKN1A (P53/P21) pathway, which induces ATM/ATR kinases recruited to the DNA damage foci [64]. Although the cells remain metabolically active, they do not proliferate [65]. At this point, somatic cells start to stimulate the transcription of genes necessary for cycle arrest, preventing the propagation of mutagenesis, and tumor development as a consequence [9]. Consequently, these cells raise senescence-induced phenotypic changes promoting a shift in their metabolism [65]. However, cells may recover their proliferative rate by TP53 gene suppression or mutation, circumventing cell cycle regulation and avoiding the senescent state [66]. In this way, the cells acquire



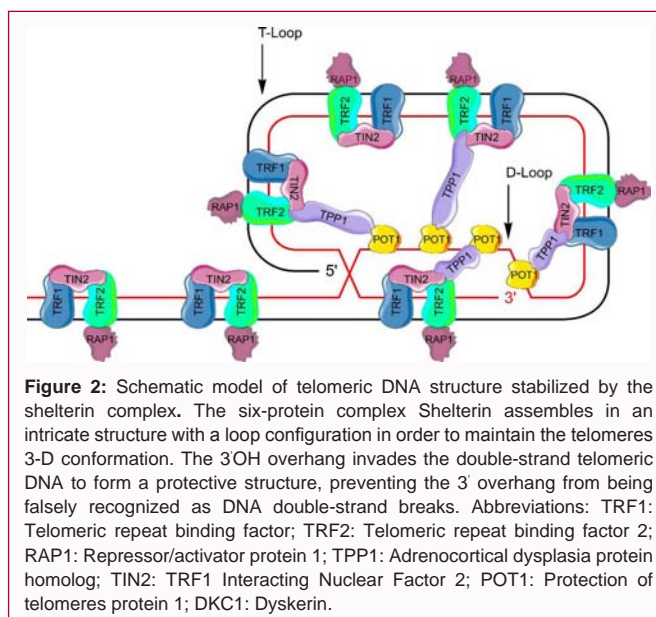
unlimited proliferative capacity, so that their telomeres shorten until reaching a critical point, being unable to protect the ends of the chromosomes. This process results in the emergence of chromosomal abnormalities, such as Homology-Directed Repair (HDR) and NHEJ, as well as anaphase bridges followed by Breakage/Fusion/Bridge (B/F/B) cycles that lead to high rates of apoptosis due to genomic instability [67,68].

Telomere dysfunction in human chronic diseases

The length of telomeres, the activity of the telomerase enzyme and the association of proteins of the shelterin complex are fundamental factors in the pathophysiology of several human diseases. Numerous studies indicate that aging-related diseases and early-age (progeria) syndromes are characterized by accelerated shortening of telomeres, which may compromise cell viability and the immunological potential through disease progression [14].

In particular, clinical evidence suggests that defect on telomeres biology and its maintenance machinery causally contributes to the establishment and progression of the aging phenotype in some diseases, termed telomeropathies. The onset and progression of these pathologies, such as Dyskeratosis Congenita (DC) [69], Aplastic Anemia (AA), Idiopathic Pulmonary Fibrosis (IPF) [70] and Hoyeraal-Hreidarsson Syndrome (HHS) are directly related to mutations on telomerase or shelterin genes and critically shortened telomeres [71,72]. Although CD, AA, IPF, and HHS are apparently different diseases with diverse clinical manifestations, they all share several characteristics, such as shortened telomeres as a causal effect. Thus, apparently heterogeneous phenotypes are caused by the same molecular defects or underlying mutations in the genes of the telomeric complex [73].

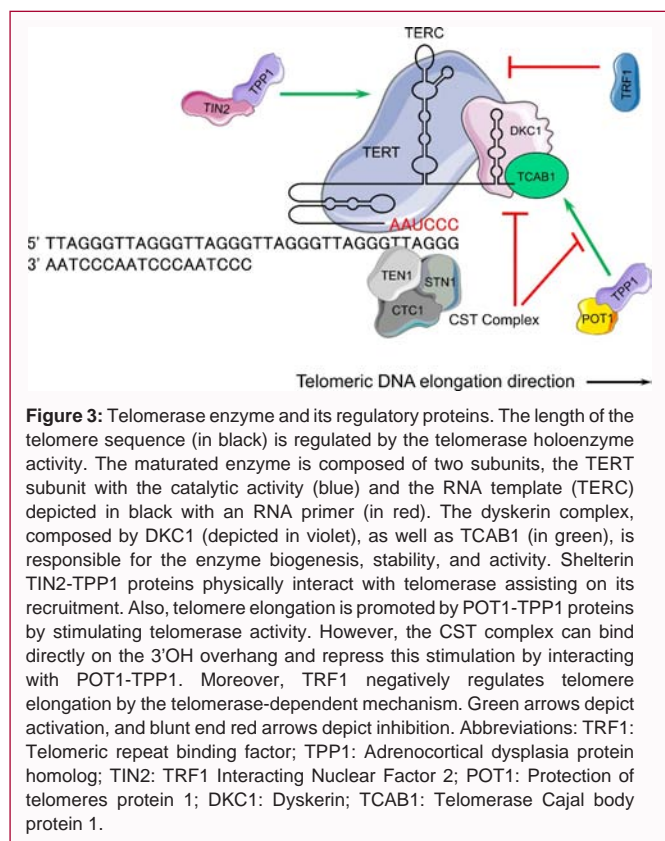
On the other hand, recent studies suggest that various chronic diseases, such as cardiovascular diseases [74], diabetes mellitus [75], metabolic syndromes [76-78], Chronic Obstructive Pulmonary Disease (COPD) and severe asthma [79-82], among others are associated with accelerated shortening of telomeres regardless of their individual biological age [70]. Chronic metabolic disorders share important characteristics and are associated with an immune system dysfunction leading to a chronic pro-inflammatory status that is closely related to the onset and persistence of such diseases [83]. These conditions are highly modulated by inflammation, oxidative stress and environmental factors, which can contribute to telomere dysfunction. The pro-inflammatory milieu triggers cellular proliferation and cell turnover, thus promoting an accelerated



shortening of telomeres. However, a pro-oxidant imbalance can also promote DSB and DDR on telomeric DNA regions, resulting in telomere shortening [70].

Increasing evidence demonstrates that comorbidities and chronic inflammation related to obesity are associated with shortened telomeres [84-86]. In this sense, we demonstrated that telomere shortening in the context of obesity is related to the condition itself, independently of comorbidities occurrences. We also observed a dysregulation on shelterin components, where TRF1 negatively contributed to telomeres' attrition [32]. Additionally, we describe chronic detrimental effects from the plasma of patients with obesity. Our findings demonstrate an immunosenescent phenotype characterized by increasing mitochondrial dysfunction and DNA damage associated with augmented apoptosis on PBMC from a eutrophic donor by supplementation with plasma from patients with obesity [87]. Longitudinal studies have focused on the effects of bariatric surgery on telomere shortening rate. In this context, Laimer *et al.* observed an increase in telomeres length in PBMC after 10 years of bariatric procedure [88]. On the other hand, Formichi *et al.* observed shorter telomeres on obese patients with no effect 12 months after intervention [89]. Literature controversy is supported by our recent review with meta-analysis comprising 119,439 patients from 39 original studies, where we demonstrate inconclusive results with a trend towards a negative correlation for obesity and telomere shortening [90].

Moreover, our studies on psychiatric diseases, such as Bipolar Disorder (BD), show shortened telomeres in individuals diagnosed with BD [91]. More interestingly, when we compared the telomere length of these individuals with their non-BD siblings and with unrelated healthy individuals; we found a progressive order of degeneration between groups. In this sense, shorter telomeres on PBMC were associated with an increased pro-inflammatory profile in BD carriers [92]. When we replicated this work in individuals with another severe neurodegenerative disease, such as Schizophrenia (SZ), we also observed increased senescence profile on cells marked by telomere attrition. However, when we analyzed the telomeres length in the same three groups, we observed that both SZ individuals and their siblings had telomeres of similar length and shorter than



unrelated healthy individuals. These results highlight a pathological profile of premature aging possibly present in the course of SZ and suggest that the length of telomeres could be an endophenotype present in individuals at risk [93]. Finally, in the last work, we demonstrated a positive association between telomere length and CCL11, a peripheral biomarker associated with inflammation in aging. We further describe negative associations between telomere length and gray matter volume or recent memory episodes in subjects with SZ [94]. Although all these studies are cross-sectional, the results of this set are consistent with the hypothesis of disease-induced accelerated disease (BD or SZ) rather than age.

Still, the reflex of the exposure to adverse situations on the length of telomeres can be detected even in childhood. Studies show an association between the shortening of telomeres and the occurrence of childhood obesity [95], as well as being related to low socioeconomic status [96] and exposure to psychological stress [97]. In a longitudinal study that followed children exposed to violence for five years, the rate of shortening of telomeres persisted, suggesting that events early in life may cause chronic alterations, increasing the risk of diseases in adulthood [98]. Other studies have shown that premature rupture of membranes is related to the shortening of leukocyte telomeres already in the fetal period [99,100]. In addition, there is evidence that adverse events during pregnancy may be associated with shortening of leukocyte telomeres in newborns [101], and in young adults [102].

Several studies have also shown that lifestyle factors, such as smoking [103], alcoholism [104], exposure to environmental pollution [105], beverage drinks consumption [106], socioeconomic status, as well as other diseases associated with chronic inflammation, and biochemical [107] or psychological stress events [108] directly influence the health and life expectancy of individuals. These might potentially accelerate the shortening rate of telomeres from PBMC,

increasing the risk of developing several types of cancer and the rate of aging, leading to a senescence phenotype or inducing cell death [14].

There is also evidence in the literature for interventions that can reverse the accelerated rate of telomeres shortening. As an example, many programs have focused on modifying individuals' comprehensive lifestyle changes. In a three-month follow-up study of patients with prostate cancer, lifestyle change, including a balanced diet, follow-up by group therapy, moderate physical activity, and control of stress levels, contributed to the elongation of the telomeric sequence, decreasing the acceleration of the aging process [109].

Conclusions

The elucidation of the complexity of replication and regulation of the structure of telomeres is a fascinating focus of basic research involving cell biology. Telomeres are cell structures associated with biological aging and are regulated by a network of complexes formed by specialized proteins, conferring stability to the sequence. Thus, telomeres are structures that play a fundamental role in chromosomal stability, avoiding the triggering of DNA damage responses to the genome of organisms, avoiding nucleolytic events during the progression of the aging process.

According to Lopez-Otín, shortening of telomeres is considered an important marker of the aging process, since this structure is highly susceptible to deterioration and related to the accumulation of DNA damage during the aging process [110]. Thus, structural or mutagenic deficiencies in the components of the shelterin complex are capable of causing a destabilization of the telomeric DNA structure, promoting structure unblocking [111] and accelerated shortening of telomeres [112].

In addition, experimental evidence suggests that telomeres shortening, DDR activation, and cellular senescence contribute to the establishment and progression of the aging phenotype. One hypothesis about aging is that abrupt shortening occurs early in the disease, suggesting that the length of telomeres may be an important biological marker in the establishment and progression of various pathologies [113].

Studies between the telomeres of cell biology and human diseases are complex. In addition, they require a deepening and holistic multidisciplinary approach in order to comprehensively understand clinically relevant data. Although the role of telomeres in cell physiology is of paramount importance during tumorigenesis, many studies have focused on much broader efforts, addressing a spectrum of complex diseases. Thus, this type of multidisciplinary approach can allow the discovery of effective therapeutic modalities and the prevention of diseases related to the acceleration of the biological aging process.

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