TELOMERE BIOLOGY: A SALIENT CONTRIBUTORY FACET OF SENESCENCE AND AGE-RELATED DISORDERS

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Abstract

Cell cycle regulation research has been extensively studied for decades, which has greatly increased our understanding of human cellular life span boundaries and their dysregulation during aging and various other disorders. The telomeres at the extremities of linear chromosomes play a crucial part in establishing these proliferative limits through mechanisms still in light. Telomeres gradually shorten with each mitosis, as predicted and frequently demonstrated. Telomerase activity in humans prevents telomeric attrition throughout embryonic development and in certain subsequent cell populations such as germline cells, stem and progenitor cells of renewable tissues, and activated lymphocytes. Specific lifestyle factors can either enhance or decrease the rate of telomere shortening. Better food and activity choices have the ability to slow the pace of telomere shortening or prevent excessive telomere attrition, resulting in delayed onset of age-related disorders and prolonged lifetime. In practice, telomeres and telomerase may be useful prognostic and screening cancer markers. Furthermore, if the reasoning is expanded to assume that telomeric attrition is the basis of cellular senescence and that accumulation of the latter equates to organismal aging, telomeres may easily explain the increasing incidence of cancer associated with human aging. This chapter discusses the significance of telomeres in aging and related diseases.

Keywords

Telomere, Telomerase, Shelterin, Aging, Cancer

Introduction

In 1973 and 1972, respectively, Soviet scientist Alexei Olovnikov and American scientist James Watson identified a fundamental issue with the replication of linear DNA molecules. Both researchers came to the conclusion that the loss of terminal DNA that must occur in order for linear DNA to replicate is problematic. The primary question that ultimately established a link between telomeres and replicative aging was how eukaryotic cells were able to overcome this end-replication issue [1, 2]. Telomeres are heterochromatic regions made up of TTAGGG repeats, which are repeating DNA, bound to a variety of specific proteins. Telomere protection depends on both the length of telomere repeats and the stability of telomere-binding proteins. Additionally, a number of epigenetic alterations govern telomere length and integrity, indicating higher order control of telomere function. Loss of telomeric protection can come from telomere lengthening below a predetermined threshold and/or modifications to the function of the telomere-binding proteins, which can cause end-to-end chromosome fusions, cell cycle halt, and/or apoptosis. Telomeres also have other roles, such as guaranteeing proper chromosomal segregation during mitosis and suppressing the transcription of genes that are near to the telomeres (a process known as subtelomeric silencing).

Telomeres, which are the protecting ends of linear chromosomes, shorten over time. Telomere shortening has been proposed as the key molecular reason of aging. Short telomeres inhibit stem cell proliferation, reducing their ability to regenerate tissues and triggering the development of age-related illnesses. Mutations in telomere maintenance genes are linked to telomere syndromes, which include Hoyeraal-Hreidarsson syndrome, dyskeratosis congenita, pulmonary fibrosis, aplastic anemia, and liver fibrosis. Telomere shortening causes chromosomal instability, which can lead to cancer in the absence of functioning tumor suppressor genes. Furthermore, mutations in telomere length maintenance genes and shelterin components, the protein complex that protects telomeres, have been linked to several forms of cancer. These observations have given hope for the discovery of therapeutic solutions to cure and prevent telomere-associated disorders, notably aging-related diseases, including cancer.

Telomere: Structure and Function

The nucleoprotein structure at the ends of the chromosomes functioning as a protective cap are telomeres (Fig. 1). These structure ensure the chromosomal integrity and stability, keeping the genetic information intact enabling the cells to multiply. Additionally they act as sites for recombination events and transcriptional silencing and also govern chromosome association

and organisation in the nucleus. A telomeric specific reverse transcriptase enzyme, telomerase, produces and adds telomeres independently of normal DNA synthesis. The telomeres of vertebrates are around 1.5 to 150 kilobases from which 50 base pairs are lost per year in humans. Very much like the rest of the chromosome the telomeric DNA is also organised into nucleosome particles, with a difference that they lack H1 histone. The DNA sequence of telomeres is what stands out the most because they are conserved through the phylogenetic tree and shows the organisms that are distantly related. Telomere length loss eventually triggers controlled cell senescence and apoptosis in healthy cells as a way to clear the DNA that would be more susceptible to mutation or altered expression and could subject the cell to be a cause of an infection, cancer, or other disease.

The double-stranded TTAGGG repeats that make up human telomeres are 4–12 kb in length, and they are followed by a single-stranded 3 overhang that is a few hundred nucleotides long. The telomeric DNA is modified into a T-loop (telomere loop) structure by the specific shelterin complex. Telomeric repeat binding factor 1 (TRF1), TRF2 (TRF2), TIN2 (TRF1-interacting nuclear factor 2), RAP1 (repressor activator protein 1), TPP1, and POT1 (protection of telomere 1) are examples of shelterin subunits. Ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and poly(ADP-ribose) polymerase 1 (PARP1)) DNA damage signaling, as well as DNA repair by c-NHEJ (canonical nonhomologous end joining), alt-EJ (alternative end joining), and HR (homologous recombination).

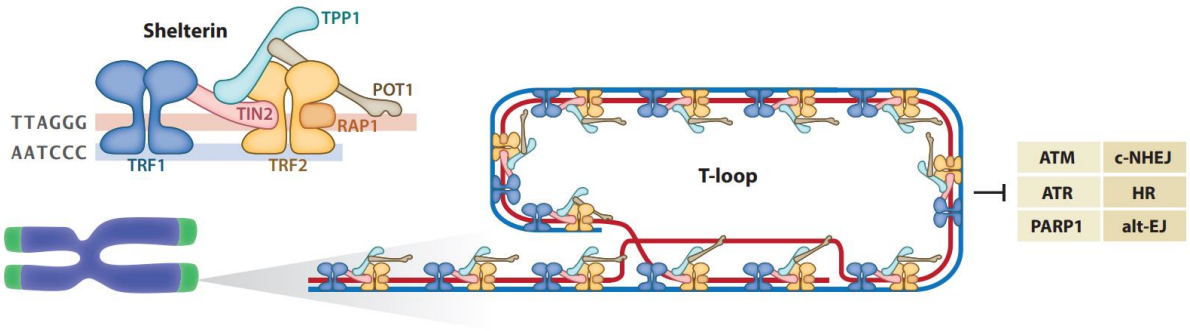

Figure 1: Overview of telomere composition and function. [3]

The Shelterin complex and its functions

Shelterin binds to mammalian telomeres and controls a number of telomere functions there (Fig. 2) [4, 5]. Telomeric repeat-binding factor 1 (TRF1), telomeric repeat-binding factor 2 (TRF2), repressor/activator protein 1 (RAP1), TRF1-interacting nuclear factor 2 (TIN2), TPP1

(also known as adrenocortical dysplasia protein homologue), and protection of telomeres 1 (POT1) are the six subunits that make up the complex known as shelterin. Through TRF1 and TRF2, which bind to TIN2 and double-stranded telomeric DNA, shelterin is attracted to telomeres. POT1 binds to single-stranded telomeric DNA, and through its binding partner TPP1, which interacts with TIN2, POT1 is connected to TRF1 and TRF2. RAP1 and TRF2 are connected.

By limiting telomerase's access to chromosome ends, preventing end-resection at freshly replicated telomeres, and shielding telomeres from the DNA damage response (DDR), shelterin maintains telomere length and protects genome integrity. Particularly, TRF2 inhibits both classical non-homologous end joining (c-NHEJ) and ATM-dependent DNA damage signaling, whereas POT1 inhibits ATR signaling and collaborates with RAP1 to inhibit homologous recombination. Through the development of TRF2-dependent t-loops, the DDR is avoided in part. T-loops are thought to prevent ATM activation by masking the chromosome end from the double-strand breaks sensor complex MRE11-RAD50-NBS1 (MRN) and by preventing the loading of the Ku70-Ku80. T-loops are formed through the invasion of the 3 overhang at the telomere end into double-stranded telomeric DNA (FIG. 1). Replication protein A (RPA), a single-stranded DNA sensor, is occluded during POT1's repression of ATR signaling. Importantly, this repression is dependent on POT1's interaction with TPP1 to link it to the rest of shelterin. When telomeres get too short to sustain enough shelterin binding, telomere protection is weakened.

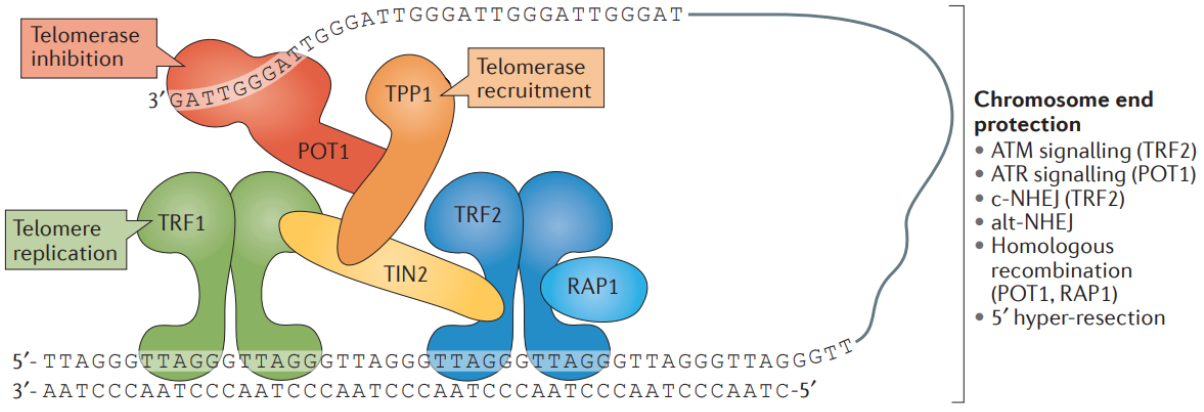

Figure 2: The Shelterin Complex [3]

Shelterin also helps the reverse transcriptase complex telomerase (FIG. 1), which is attracted to telomeres by the components of shelterin TPP1 [6, 7, 8] and TIN2 [9], maintain telomeres [10]. Shelterin subunits may 'count' telomeric repeats to control telomerase activity and limit

telomere length as part of a cis-acting negative-feedback loop since some shelterin subunits are negative regulators of telomere length [11]. This regulatory system may be significant in the germ line, where telomere length must be kept within a specific range to ensure that children have telomeres long enough for normal development and tissue homeostasis, but at the same time, telomere length needs to be maintained within a small range in the germ line.

Telomeric DNA, the shelterin complex, and the telomerase complex make up the three parts of a human telomere (Fig. 3). A long series of double-stranded TTAGGG repeats make up the telomeric DNA, which ends in a 50–300 nucleotide single-stranded 3 overhang. This 3′ Double-stranded telomeric repeats are invaded by overhang to create a t-loop structure that is essential for telomere function. The six-subunit shelterin complex interacts with telomeric DNA to protect chromosomal ends. Telomerase, which consists of telomerase reverse transcriptase (TERT), telomerase RNA template component (TERC), and various auxiliary proteins (blue), can maintain the length of telomeric repeats. TERT uses TERC as a template to create telomeric DNA from scratch, whereas auxiliary factors are involved in telomerase's biosynthesis and nuclear transport. NHP2, a non-histone protein 2, NOP10, a nucleolar protein 10, POT1, a protein that protects telomeres, RAP1, a protein that represses or activates gene transcription, TCAB1, a protein that is part of the telomerase Cajal body, TIN2, and TRF, a protein that binds to telomeric repeats.

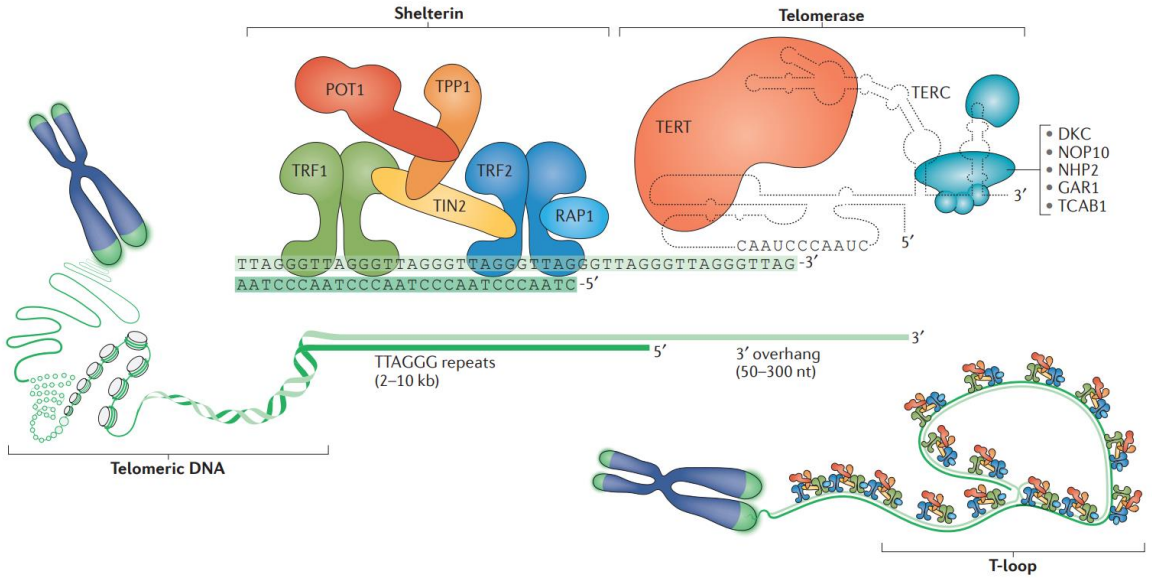

Figure 3: Composition and structure of the human telomere system. [3]

Telomere length: A biomarker for aging and techniques to measure it

Long held theories suggest that telomere length and telomere shortening are biological indicators of ageing at the cellular level [12]. There are various techniques which can be used to detect the length of the telomeres such as, quantitative PCR (qPCR), southern blot, flow cytometry with fluorescence *in-situ* hybridization (Flow-FISH), single or universal single telomere length analysis (STELA), quantitative FISH (Q-FISH). The most commonly used methods are the southern blot and q-PCR which are employed by the epidemiological studies of the association of the telomere length with the carcinogenesis and studies detecting the effects of the environmental factors on the length of telomeric sequences. In the southern blotting technique high quality DNA is subjected to digestion by the restriction endonucleases enzymes which cuts the DNA into smaller fragments [13]. These selective endonucleases do not bind to the telomeric and the related regions. Therefore when the electrophoresis gel is run, the large sized telomeric DNA are separated and are detected under the chemiluminescent or radio-labelled probe for the terminal restriction site. Southern blot generates results that include the subtelomeric (noncanonical) parts of the telomere and requires a substantial amount of DNA (at least 3 mg), which may overstate the length of the telomere. Additionally, the type of restriction enzyme used to digest the DNA can affect reproducibility. On the bright side, due to its reproducibility and ability to report results as the actual length of the telomere in terminal restriction fragment (TRF) units, Southern blot analysis is regarded as the gold standard for telomere length measurement. This enables comparison of telomere length (actually) TRF between various studies [14, 15].

Quantitative polymerase chain reaction (qPCR) is the method most frequently employed to measure telomere length in cancer risk association studies. This PCR-based assay amplifies the telomere by using a set of primers targeting the telomeric hexamer repeats [16, 17]. The canonical telomere length, excluding the subtelomeric region-FISH, is detected and finally measured by both Flow FISH and Q-FISH using fluorescently labeled peptide nucleic acid probes. Flow-FISH combines in situ hybridization and flow cytometry to assess the average telomere length across all chromosomes but of specific cell subsets, such as granulocytes and lymphocytes from a peripheral blood sample [18]. This can be done on the nuclei of intact cells that are in suspension or have been frozen. To determine metaphase, Q-FISH needs cells that are actively dividing or a chromosomal spread, and it can measure the telomere length of a specific chromosome rather than just the average telomere length of all the chromosomes [19]. STELA combines electrophoresis-based DNA fragment separation, Southern blotting

hybridization, and PCR amplification to precisely measure even extremely small telomere segments at a single chromosomal end. Even at the single cell level, this method can assess chromosome-specific telomere lengths from smaller amounts of DNA than qPCR, but only for the chromosomes for which particular probes have been created. As an average measurement across all chromosomes, Universal STELA integrates the accurate measurement and capacity to identify very short telomeres [20]. Both of these methods need more work and time than qPCR, but they quantify canonical telomere length more precisely [21]. Telomere erosion is an appealing choice for research that seek to explain the rise in incidence of various malignancies with advancing age since it is a process that is related to ageing at the cellular and tissue level and is also implicated in carcinogenesis.

Telomere induced senescence

Telomere length reduces with age as a normal cellular mechanism [22]. Human telomere length appears to be decreasing at a rate of 24.8-27.7 base pairs per year [23]. Telomere length that is shorter than the average for a certain age group has been linked to an increased prevalence of age-related disorders and/or a shorter lifespan in humans [24, 25, 26]. A variety of factors influence telomere length, including donor age [27], genetic, epigenetic make-up, and environment [28, 29], social and economic status [30, 31], exercise, body weight [32], and smoking [33, 34]. Gender does not appear to have an effect on the rate of telomere loss [35]. When telomere length falls below a certain threshold, cells enter senescence and/or apoptosis [36, 37].

Certain lifestyle factors, such as smoking, obesity, lack of exercise, and eating a poor diet, can hasten the shortening of telomeres, resulting in sickness and/or premature mortality. Many age-related health concerns, including coronary heart disease [38, 39], heart failure [40], diabetes [41], increased cancer risk [42, 43], and osteoporosis, are associated with accelerated telomere shortening. Individuals with leukocyte telomeres that are three times shorter than the corresponding average telomere length are three times more likely to develop myocardial infarction [44]. Telomere length analysis in elderly people reveals that those with shorter telomeres die at a substantially higher rate than those with longer telomeres [45]. Excessive or rapid telomere shortening can have ramifications for health and longevity on several levels. Shorter telomeres can also cause genomic instability by facilitating interchromosomal fusion, and they may play a role in telomere stabilization and cancer formation [46]. Telomerase activity is consistently increased in most cancer cells, whereas telomere length is decreased in

comparison to equivalent control cells. Telomere length in cancer cell lines and primary cancer cells purified by laser capture microdissection is shorter [47]. In immortal/cancer cells, however, blockage of telomere maintenance mechanisms and continuous telomere shortening produces senescence and/or apoptosis [48, 49].

Only a few population doubles can occur in vitro for the majority of human somatic cells1. Senescence—the exhaustion of proliferative capacity—can be brought on when telomeres, the ends of linear chromosomes, are unable to perform their regular protective roles [50]. One or two chromosomal ends in a cell must lose telomere function in order to cause replicative arrest [51, 52]. The rate of telomere shortening, the initial telomere length, and, most significantly, the length of the shortest telomeres in the cells all play a role in when telomere attrition causes the loss of telomere protection at one or more chromosomal ends. Measurements of bulk telomere length are a poor predictor of cellular proliferative potential because human telomeres are heterogeneously shaped and can contain numerous very short telomeres in cells with an apparent adequate telomere reserve.

According to several studies, shorter telomeres are a risk factor for cancer. Shorter telomeres appear to increase the chance of developing lung, bladder, renal cell, gastrointestinal, and head and neck cancers [53, 54]. Certain people may be born with shorter telomeres or have a genetic condition that causes shorter telomeres. These people are more likely to develop premature coronary heart disease [55, 56] and premature aging. Shorter telomeres are related with premature graying, propensity to cancer, vulnerability to infections, progressive bone marrow failure, and premature death in adults in the hereditary condition dyskeratosis congenital [57].

Telomere in cancer

By preventing chromosome ends from being misinterpreted for DNA damage, telomeres help cells continue to divide and retain the integrity of the genome. The telomeric shelterin complex, which inhibits DNA damage signaling and repair pathways, provides chromosome end protection. Human telomere shortening during the development of cancer has two conflicting impacts. One way that telomere shortening can reduce tumor growth is by causing a proliferative arrest at exposed chromosomal ends by activating the kinases ATM and ATR. On the other side, telomere crisis—a state of extreme genomic instability—can accelerate the development of cancer if telomere protection is lost. Due to inadequate DNA replication and exonucleolytic processing, telomeres shrink during cell proliferation in telomerase-negative cells. By impairing telomere function, attrition causes cell cycle halt, senescence or death,

signaling by the kinases ATM and ATR, and telomere deterioration. Telomeres, the very apparatus that protects chromosomal ends and prevents false rearrangements, also serve as substrates for the same process as time passes (in the replicative sense). Concurrently, shortened telomeres cause senescence, reducing the build-up of aberrations within the gene pool. On the other side, if senescence is skipped, the same condition results in genomic instability, which leads to oncogenesis.

On univariate analysis, the presence or amount of telomerase activity does not appear to correlate with progression-free survival in cervical cancer. The presence or degree of hTERT mRNA expression is also unrelated to progression-free survival [58]. In studies of telomerase activity in cervical cancer screening as a potential diagnostic marker, its sensitivity in detecting cervical intraepithelial neoplasia (CIN) grade II/III lesions has been reported to be low, especially when compared to detecting high risk HPV (human papillomavirus) [59]. High telomerase activity appears to be associated with tumor recurrence and a lower 5-year overall survival rate in colorectal cancer [60]. Immunohistochemical detection of hTERT in tumors is associated with decreased median survival for patients with metastatic colorectal cancer in the liver, and multivariate analysis demonstrates that positive hTERT staining is an independent predictor of poor survival [61].

Telomerase activity is a strong predictor of both disease-free and overall survival in non-small cell lung cancer (NSCLC) [62, 63]. Strong hTERT mRNA expression detected by ISH, on the other hand, is associated with a worse 5-year survival rate and is a significant prognostic factor [64]. Patients with hTERT mRNA ISH have considerably poorer disease-specific and disease-free survival rates [65]. The detection of hTERT mRNA in spontaneously voided urine by RT-PCR appears to be more sensitive than urine cytology in the diagnosis of bladder cancer, and the specificities of the two assays are comparable [66].

The overall trend of findings in this context suggests that telomerase activity is connected to disease severity, particularly Clark level [67] and metastatic dissemination [68, 69, 70]. However, the sample size of each of these trials is too small to draw any firm conclusions, and none of them provide patient survival data.

Individual investigations of telomerase evaluation in prostate cancer lack comparative statistical rigor with small sample sizes and do not include telomerase measurement in the context of survival outcome analysis. However, the reported findings point to telomerase activity [71, 72] and hTERT expression [73, 74, 75, 76] being unrelated to illness stage.

Positive hTERT immunostaining is more common in malignant mesotheliomas than in benign mesotheliomas, implying that hTERT immunohistochemistry may be beneficial in differentiating these two diseases [77].

Entities linking telomeres to aging and cancer

If telomeres are the link between aging and cancer, then this link may be more visible in illnesses of premature aging. Three such entities are described below: Werner's syndrome, Bloom's syndrome, and dyskeratosis congenita. Each of these illnesses is caused by mutations that appear to impair telomerase activity.

Werner's syndrome, a premature aging disorder with a susceptibility to cancer [78], is caused by mutations in WRN, a RecQ family DNA helicase with extra exonuclease and ATPase activity [79].

BLM [80], another member of the RecQ helicase family, is mutated in Bloom's syndrome [81]. The latter presents as dwarfism, reduced fertility, immunodeficiency, photosensitive face erythema, and, once again, a proclivity for cancer [82]. At the telomeres, a fraction of the cellular pool of WRN co-localizes and associates with TRF2 [83].

DC is distinguished by a rapidly aged phenotype with mucocutaneous alterations, an early death caused mostly by bone marrow failure, and a proclivity for chromosomal rearrangements and malignancy [84]. Its X-linked recessive variant is caused by mutations in dyskeratin [85], a component of the box H/ACA small nucleolar (sno) RNA ribonucleoprotein particle (RNP) [86].

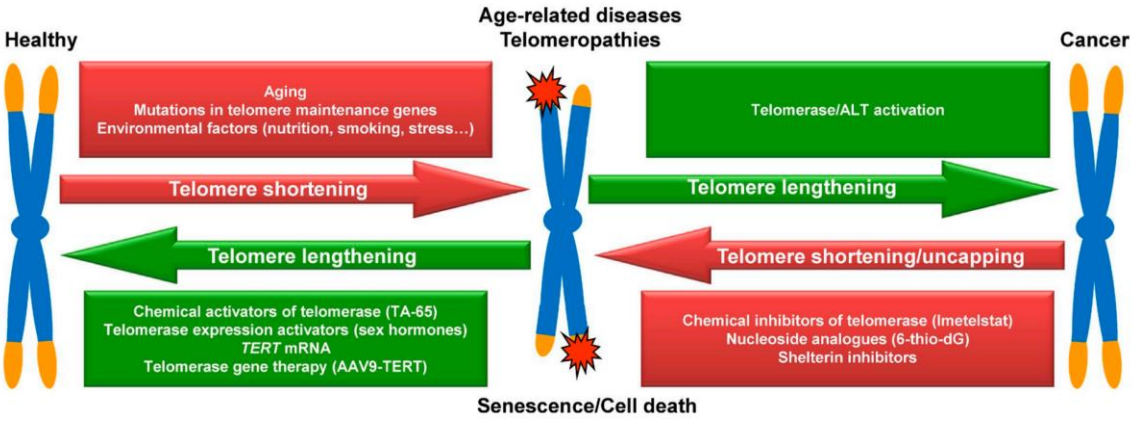

Figure 4: Natural and therapeutic variables influencing telomere-mediated illnesses [87]

Telomere shortening happens spontaneously throughout life as a result of cell division, the rate of which can be modified by genetic and environmental variables (Fig. 4). Short, unprotected

telomeres trigger a DDR that causes cellular senescence, impairing tissue regeneration potential and giving rise to a slew of age-related disorders, including the so-called Alzheimer's disease. Telomeropathies are conditions in which tissue deterioration begins prematurely due to hereditary telomere maintenance abnormalities [88]. To combat telomere shortening, several therapeutic interventions are being investigated, including chemical telomerase activators (TA-65), activators of telomerase reverse transcription (TERT) transcription (sex hormones), intracellular administration of TERT mRNA, and telomerase gene therapy (AAV9-TERT). Spontaneous mutations that activate telomerase expression, or ALT, result in telomere lengthening, which allows cells with genetically fragile checkpoints to proliferate indefinitely and eventually become cancer cells [89]. Several anticancer treatment methods based on chemical stimulation of telomere malfunction have been evaluated. Among other things, we emphasize the use of a telomerase inhibitor (imetelstat), a nucleoside analogue (6-thio-dG), and compounds that inhibit shelterin components [90].

Telomeres in young, healthy cells are long and fully protected, but they shrink with increasing cell divisions due to the end replication problem, replication fork collapse, nucleolytic processing, and oxidative stress (Fig. 5). This gradual telomere shortening eventually results in certain dangerously short deprotected telomeres known as intermediate-state telomeres. Telomeres maintain enough shelterin to block fusions but cause a DDR marked by the creation of the so-called telomere-induced focus (TIF) in this intermediate stage of deprotection. When at least five telomeres become dysfunctional (more than five telomere-induced foci), a DDR marked by p53 activation is triggered, resulting in replicative senescence [91]. Telomere attrition in stem cell compartments reduces tissue and self-renewal ability and is thought to be a main biological reason. Telomeropathies, also known as telomere syndromes, occur when telomere attrition occurs prematurely as a result of germline abnormalities in genes coding for telomere maintenance and repair proteins. Successive telomere shortening across generations demonstrates genetic anticipation, with illnesses onset occurring at an earlier age and symptoms worsening [92, 93]. Senescence can be avoided by acquiring loss-of-function mutations in the p53 and p16/Rb tumor suppressor genes, which allow for continued proliferation during the extended life span period, during which cells experience further telomere shortening and eventually enter the uncapped state, where they lose all protective properties and fuse [94]

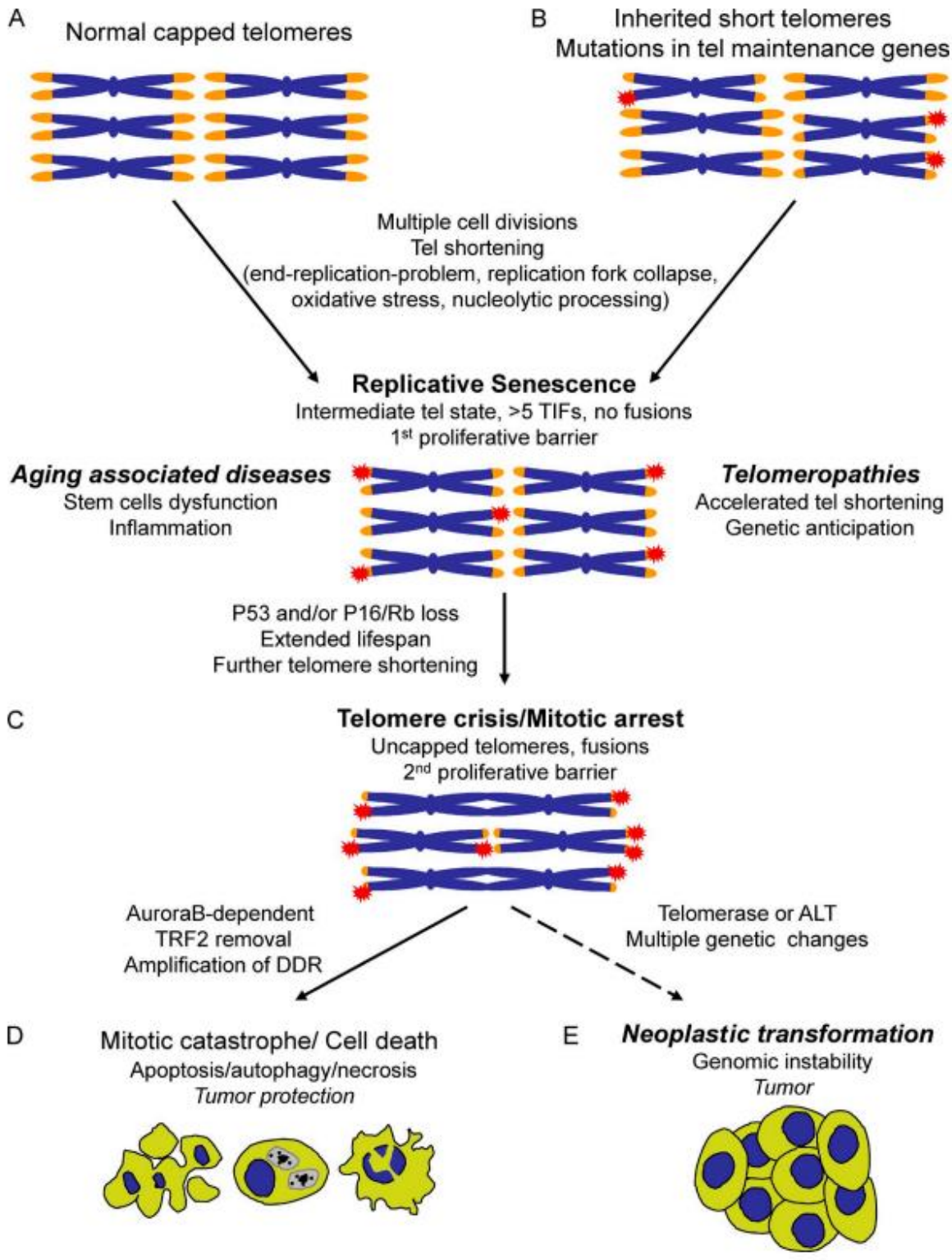

Figure 5: Telomere shortening and its impact on aging-related disorders, telomeropathies, and cancer [87]

Fused telomeres trigger a mitotic arrest checkpoint, during which telomere dysfunction is increased by Aurora B-dependent TRF2 elimination, resulting in cell death via apoptosis,

necrosis, or autophagy in crisis, a second proliferative barrier that prevents tumor formation [95]. In some very rare crisis cells, reactivation of either telomerase activity or ALT permits these premalignant cells to escape crisis and divide indefinitely (immortalization). Fused chromosomes in postcrisis cells cause large-scale genomic rearrangements, which enhance the acquisition of oncogenic mutations and malignant features needed for a fully malignant phenotype, cancer [96].

Conclusion

Telomeres shorten as we become older, and progressive telomere shortening causes senescence and/or apoptosis. Telomere length has also been linked to genomic instability and oncogenesis. Elderly persons with shorter telomeres are three and eight times more likely to die from cardiovascular and infectious diseases, respectively. The rate of telomere shortening is so essential to an individual's health and aging. Tobacco use, pollution exposure, a lack of physical activity, obesity, stress, and a poor diet all raise the oxidative burden and pace of telomere shortening. We may explore eating less to retain telomeres and minimize cancer risk and the rate of aging; including antioxidants, fiber, soy protein, and healthy fats (derived from avocados, fish, and nuts) in our diet; and staying slim, active, healthy, and stress-free through regular exercise and meditation. Foods high in antioxidants include tuna, salmon, herring, mackerel, halibut, anchovies, catfish, grouper, flounder, flax seeds, chia seeds, sesame seeds, kiwi, black raspberries, lingonberry, green tea, broccoli, sprouts, red grapes, tomatoes, olive fruit, and other vitamin C and E-rich foods. These, together with a Mediterranean-style diet rich in fruits and whole grains, would help protect telomeres.

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