The Aging Mechanism and the Telomere Depletion Theory

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Abstract. Aging in mammals is a complex biological process which is influenced by multiple mechanisms, and telomere attrition is recognized as a key hallmark among them. The gradual reduction of telomeres, which are protective structures located at the ends of chromosomes, is closely associated with cellular senescence and organismal aging. However, the precise contribution of telomere dynamics to aging and age-related diseases still has some uncertainties, and it is still debated whether telomere shortening is a cause or consequence of these processes. Recent studies have focused on both genetic and environmental factors affecting telomere length, as well as the molecular mechanisms of telomere maintenance and dysfunction. This article comprehensively outlines nine major mechanisms of aging and has a particular focus on the structure, function, and regulatory mechanisms of telomeres. The article also emphasizes the mechanism of telomere shortening and its impact on cellular and tissue aging. Additionally, the special roles of telomere maintenance mechanisms in stem cells and cancer cells are discussed. Regarding the shortening and maintenance mechanisms of telomeres and telomeres within cells, the article introduces some recent anti-aging and anti-cancer techniques, enabling readers to have a more comprehensive understanding of the role and prospects of telomeres in these fields.

Keywords: Aging; Telomere; Cancer; Telomerase; CRISPR.

1. Introduction

The internationally recognized "Nine Hallmarks of Aging" are widely acknowledged among multiple aging mechanisms. The theory systematically summarizes various aging mechanisms, including genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, loss of proteostasis, deregulated nutrient sensing, cellular senescence, stem cell exhaustion, and altered intercellular communication [1]. In addition to these endogenous factors, exogenous factors such as unhealthy lifestyles [2] can also exert detrimental effects. The damage caused by aging, such as functional decline and organ failure, still being difficult to reverse at present. Therefore, investigating the nature of aging and anti-aging methodologies is of significant interest.

Among the mechanisms that are mentioned earlier, telomere attrition plays a decisive role in cellular aging. Telomeres are composed of telomeric DNA and associated proteins, establishing highly conserved tandem repeat sequences (5'-TTAGGG-3' in humans). These sequences primarily exist as double-stranded regions (10–15 kb in humans) but terminate with a short, single-stranded, G-rich 3' overhang (50–500 nt in humans). Telomere length varies across different tissues and organs in the human body [3], and both genetic and exogenous factors have an impact. Telomeres progressively shorten as cells undergo continuous division, eventually triggering DNA damage responses (DDR), which can lead to cell cycle arrest, cellular senescence, or apoptosis. Telomere length cannot be maintained permanently by these processes permanently although telomeres having inherent structural capabilities to preserve their length. In contrast, tumors and cancer cells exploit specialized mechanisms to prevent telomere shortening, thereby contributing to the development of severe diseases.

Aging and cancer have been challenging issues for humanity over time. Telomeres offer numerous avenues for anti-aging and cancer therapies for the relationship between structure and both processes, including CRISPR-mediated regulation of telomerase gene expression, augmentation of telomere-protective proteins, and partial reprogramming methodologies utilizing Yamanaka factors (OSKM), which are all aimed at telomere maintenance from diverse angles.

This article will first introduce the structure of telomeres and the factors influencing their length, followed by an explanation of the impact of telomere attrition on cells and their maintenance mechanisms. The special techniques used by cancer cells to control telomere length will also be covered. Finally, the article will discuss various cutting-edge research directions in telomere-based anti-aging strategies.

2. Telomeres and Aging

2.1. Factors influencing telomere length

Telomere length decreases with aging at a rate of about 20–40 bp annually, with cells dividing more often experiencing faster attrition. However, this rate is not fixed. The determinants of telomere length can be categorized into two types: genetic factors and non-genetic factors [4].

Epidemiological studies have identified nine loci significantly associated with telomere length, and these loci affect the length of telomeres in human tissues by controlling the expression of known telomere-maintenance genes and additional genes implicated in telomere maintenance [5, 6]. A systematic investigation analyzes telomere length changes across various human tissues during aging and their potential roles, using the Genotype-Tissue Expression (GTEx) project. It revealed that telomere length differences primarily originate from germ cells [3]. Furthermore, variations in telomere shortening between sexes due to differences in telomere attrition rates and the degree of shortening are influenced by many factors such as hormone levels, lifestyle, body composition, and telomere dynamics on the X and Y chromosomes. For example, androgens increase metabolic burden and oxidative stress levels, and that accelerates telomere attrition. In contrast, estrogens enhance antioxidant defenses to reduce DNA damage, which is caused by free radicals, thereby indirectly protecting telomeres. Studies indicate that women of reproductive age experience significantly slower telomere attrition compared to men of the same age. However, postmenopausal women show telomere shortening rates closer to those of men, which is likely due to declining estrogen levels. Additionally, compared to the Y chromosome in males, females benefit from having two X chromosomes, which provide a "protective mechanism," and it potentially offers better compensation for telomere attrition [7, 8]. These findings highlight the genetic influences on telomere attrition. Beyond genetic factors, various endogenous factors also affect telomere length, such as the end-replication problem, oxidative stress, inflammation, and genetic composition [9], as well as numerous exogenous factors, such as lifestyle [2]. Therefore, healthy exercise routines, proper sleep, and balanced diets similarly play significant roles in combating aging.

2.2. The shortening of telomeres and the maintenance mechanism

In normal cells, with each cell division, the incapacity of DNA polymerase to fully replicate the 3' terminus of linear chromosomes results in the progressive loss of telomeric DNA repeat sequences during each round of DNA replication. Consequently, telomeres gradually shorten, and without telomere maintenance mechanisms, this process continues uninterrupted. Shortened telomeres trigger DNA damage responses (DDR), which lead to DNA double-strand break signaling. The signal will cause cellular growth arrest, during which cells attempt to repair the damage. If such terminal DNA damage cannot be repaired, replicative senescence is triggered [10]. To maintain their length, telomeres form various specialized structures. Guanine-rich telomeric DNA readily forms G-quadruplex (G4) structures, which regulate telomerase activity and play critical roles in DNA replication, transcription, and telomere maintenance, in turn influencing telomere length and cellular lifespan [11]. Additionally, the ends of chromosomal telomeres are protected by forming a T-loop structure. This occurs when the 3' single-stranded overhang of telomeric DNA invades the homologous double-stranded TTAGGG repeats, forming a looped structure that neutralizes free radicals and protects the telomere [12]. Beyond these structural features, telomeric DNA also interacts with the shelterin complex, a six-protein complex that includes core components essential for telomere maintenance and DNA expression regulation [5, 13]. Chromosome ends are protected by

DNA damage repair mechanisms and regulating telomerase activity. The structural feature prevents the linear chromosome ends from being recognized as DNA damage requiring repair by inhibiting the ATM and ATR signaling pathways, thereby shielding the chromosome ends from unwanted interference by cellular DNA repair mechanisms. Unprotected shortened telomeres (uncapped telomeres) activate the DDR system [14], causing them to be mistaken for DNA double-strand breaks (DSBs). At these sites, DNA damage markers such as γ-H2AX and 53BP1 accumulate, which leads to cell cycle arrest at the G1/S or G2/M phases. Furthermore, telomere-telomere fusions may occur, resulting in cellular senescence and impairing tissue regeneration capacity. Senescent cells also secrete various inflammatory cytokines, proteases, and growth factors, collectively known as the senescence-associated secretory phenotype (SASP). These secretions affect neighboring cells and the tissue microenvironment, in turn promoting chronic inflammation, tissue fibrosis, and functional decline [3]. A range of age-related diseases and so-called telomeropathies are contributed by this cascade of events.

2.3. The anti-aging mechanism of tumor cells and its inspiration

Unlike normal somatic cells, certain self-renewing cells, including stem cells and the majority of cancer cells, overcome telomere shortening-induced senescence or apoptosis through telomere maintenance mechanisms.

In cancer development, cells that become immortal often activate telomerase, a nucleoprotein complex [10]. The protein TERT (telomerase reverse transcriptase) forms a complex with another critical component, telomerase RNA component (TERC), along with accessory proteins such as dyskerin (DKC1), TCAB1, NHP2, NOP10, and GAR1. This complex uses its RNA as a template to synthesize telomeric DNA repeats via a reverse transcription mechanism, which can add these repeats to the ends of telomeres to counteract the end-replication problem and prevent telomere shortening [15]. Telomerase activity is closely associated with tumorigenesis [16], cell proliferation, and cellular senescence [17]. In normal human cells, alternative splicing of TERT may inhibit telomerase activity during development, and the maximum length of human telomeres is limited consequently [18]. However, the regulatory factors governing TERT splicing mechanisms remain unclear, and other mechanisms may also contribute to telomerase regulation.

Similarly, CRISPR technology can be used to target and edit the TERT gene, and it encodes telomerase reverse transcriptase. In senescent cells, CRISPR can be used to precisely edit DNA or RNA targets to express telomerase [19]. This reactivation can lengthen telomeres, which can enhance the lifespan and functional capacity of these cells [20]. Conversely, CRISPR's base-editing capabilities can be utilized to reduce TERT transcription and protein expression to induce senescence and proliferation arrest in cancer cells [21]. It is worth noting that the specificity of CRISPR components must be improved. To minimize risks, rigorous screening methods should be adopted to detect and reduce off-target effects.

Additionally, Yamanaka factors, particularly Klf4, can directly bind to the TERT promoter, which can activate telomerase expression and thereby extend telomeres [22]. The reprogramming mediated by these factors can reshape the structure and function of telomeres and then restore them to a more youthful state. Moreover, Yamanaka factors activate telomerase, which results in telomere elongation and significantly enhances the proliferative capacity of iPSCs during the generation of induced pluripotent stem cells (iPSCs) [23].

In addition, a DNA homologous recombination mechanism, known as the alternative lengthening of telomeres (ALT), is activated in approximately 10–15% of tumors. ALT cells utilize a telomeric DNA template, copying to the telomeres of non-homologous chromosomes. This mechanism may involve circular or even linear extrachromosomal telomeric DNA [10]. Through this process, telomeric DNA can form a loop, adding TTAGGG sequences to a different region of the same telomere or to the telomeres of sister chromosomes. Oncogenic viral proteins can bypass cellular senescence, which allows cells to extend their lifespan despite continuous telomere shortening with this unique strategy.

ALT represents a telomere-lengthening mechanism, which operates independently of telomerase and is particularly crucial in cancer cells lacking telomerase activity. Leveraging this understanding, CRISPR technology, like deleting the TERT or TERC subunits of telomerase, can be used to activate ALT. This activation of ALT could increase telomere length in normal somatic cells, offering potential avenues for anti-aging interventions [24].

3. Conclusion

Telomeres, as critical protective structures at the ends of chromosomes, exhibit dynamic changes in length and functionality that are closely linked to aging and diseases in mammals. In addition to exploring the unique telomere maintenance strategies of cancer cells, this review summarized the molecular mechanisms of telomere attrition and its impact on cellular and tissue aging. Studies indicate that both genetic and environmental factors regulate the telomere length. Cellular senescence and apoptosis are triggered by telomere shortening, and it is associated with various age-related diseases. Given the potential of telomere maintenance mechanisms in anti-aging and cancer therapeutics, OSKM partial reprogramming and CRISPR technologies hold significant promise for telomere elongation and the restoration of cellular function.

Despite substantial progress, this field still faces many challenges. The safety and efficacy of partial reprogramming require additional refining, especially in mitigating the danger of tumorigenesis. Furthermore, the regulatory mechanisms of telomere length are not yet fully elucidated, and inter-tissue differences and the dynamic regulation of telomere length require even greater attention. In conclusion, translating telomere-related technologies into clinical applications for anti-aging and cancer treatment requires further exploration. Future research should focus on the precise regulation of telomere maintenance mechanisms and the development of safer, more efficient technologies that provide new strategies and tools for delaying aging and treating cancer.

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