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Antioxidant γ-Tocotrienol and CRISPR-Cas9-Mediated TERT Disruption in Aging and Disease Prevention

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Abstract. Telomere shortening is a hallmark of cellular aging and a contributing factor to age-related diseases. This study explores both biochemical and genetic strategies aimed at preserving telomere length and telomerase activity. Specifically, the protective effects of γ-tocotrienol—a potent antioxidant in the vitamin E family—were investigated using human dermal fibroblasts from different age groups under oxidative stress induced by hydrogen peroxide (H_2O_2). The results showed that pretreatment with γ-tocotrienol significantly protected telomeres and maintained telomerase activity, particularly in middle-aged and elderly cells, though this effect was dose-dependent. Additionally, nutritional factors such as collagen were reviewed, revealing gender-specific correlations between dietary intake and telomere length. On the genetic front, CRISPR-Cas9 technology was utilized to disrupt the TERT gene in various cancer cell lines, highlighting its potential as a tool for telomerase inhibition and functional imaging. These findings suggest that combining antioxidant therapy with gene-editing approaches may offer synergistic benefits for delaying aging and preventing telomere-related degeneration. Future research should aim to optimize treatment protocols and assess long-term safety and efficacy in clinical models.

Keywords: Telomere Shortening, γ-Tocotrienol, CRISPR-Cas9, Cellular Aging.

1. Introduction

With the rapid development of human society, demographic structures are undergoing significant changes. One of the most pressing issues is population aging — the proportion of elderly individuals is steadily increasing worldwide. Aging, in a biological sense, refers to the gradual decline in physiological and functional capacities after an organism reaches maturity [1]. In a societal context, it implies a growing demand for healthcare resources and poses challenges to sustainable social and economic development [1,2].

On the molecular level, aging is closely linked to genomic instability, particularly the progressive shortening of telomeres — specialized nucleoprotein structures located at the ends of chromosomes. Telomeres protect genomic DNA during replication by preventing the loss of essential genetic information [3]. However, with each cell division, telomeres become progressively shorter because DNA polymerase cannot fully replicate the 3' ends of linear DNA. Once telomeres reach a critically short length, cells enter senescence or apoptosis, contributing to aging and age-related diseases.

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Telomerase, a ribonucleoprotein complex composed of a catalytic subunit (TERT) and an RNA template (TERC), can counteract telomere shortening by adding telomeric repeats to chromosome ends. TERC is generally expressed in all cells, but telomerase activity is usually low or absent in most somatic cells [4]. Recent studies suggest that oxidative stress — a condition that intensifies with age — accelerates telomere shortening, further exacerbating cellular aging. Compounds like Coenzyme Q10 and γ-Tocotrienol have been investigated for their antioxidant effects and potential roles in modulating telomere length and telomerase activity [3,5].

One study used human dermal fibroblasts from three age groups (young, middle-aged, and elderly) and examined the protective effects of γ-Tocotrienol against oxidative damage induced by hydrogen peroxide (H_2O_2) at IC_{50} concentrations. Techniques such as the MTS assay, Southern blotting, and Telomere Repeat Amplification Protocol (TRAP) were employed to assess mitochondrial function, telomere length, and telomerase activity, respectively. Results indicated that γ-Tocotrienol did not significantly affect telomere length or telomerase activity in young and elderly cells under oxidative stress [6].

In addition to pharmacological approaches, gene editing technologies like the CRISPR-Cas system offer a promising avenue to directly modulate telomeres and telomerase genes. This system consists of a single guide RNA (sgRNA) that directs the Cas9 protein to specific DNA sequences for precise genome editing. Cas9, a widely used endonuclease, can be employed to regulate genes involved in telomere maintenance, offering potential therapeutic strategies for age-related disorders [7,8].

Therefore, this article aims to explore the molecular mechanisms underlying telomere shortening and aging, examine recent therapeutic strategies — including antioxidants and CRISPR-based gene editing — and evaluate their potential in preserving telomere length and delaying cellular aging.

2. Tocotrienols and their protective effects on oxidative stress-induced cellular damage

Tocotrienols, members of the vitamin E family, are structurally similar to tocopherols, with the primary difference being the saturation of their side chains. While tocopherols contain a saturated phytyl tail, tocotrienols possess an unsaturated isoprenoid side chain. This structural distinction significantly influences their biological activity. The presence of the unsaturated prenyl side chain is believed to enhance tocotrienols' ability to penetrate cellular membranes, thereby altering their distribution and metabolism compared to tocopherols. This feature is also thought to contribute to their superior antioxidant properties, which are of particular interest in the context of aging and oxidative stress [9].

To investigate the protective effects of γ-tocotrienol against oxidative stress-induced damage, a series of experiments were conducted using human skin fibroblast cells derived from individuals across three age groups: young, middle-aged, and elderly. The cells were first exposed to increasing concentrations of hydrogen peroxide (H_2O_2) to induce oxidative stress and determine the IC_{50} values —the concentration required to reduce cell viability by 50%. The results revealed age-dependent sensitivity to H_2O_2, with fibroblasts from young individuals exhibiting an IC_{50} at 700 μM, while those from middle-aged and elderly individuals showed IC_{50} values at 400 μM and 100 μM, respectively. At even higher concentrations—1000 μM for young, 900 μM for middle-aged, and 800 μM for elderly fibroblasts—almost all cells lost viability, indicating that cells from older individuals are more vulnerable to oxidative damage [10].

The study then evaluated the effects of γ-tocotrienol on cell viability under oxidative stress. When applied alone, γ-tocotrienol demonstrated a clear dose-dependent effect. Pretreatment with γ-tocotrienol for 24 hours increased the number of viable cells, particularly at optimal concentrations

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of 80 μM for young fibroblasts and 40 μM for both middle-aged and elderly fibroblasts. However, as the concentration increased beyond these optimal levels, a significant decline in cell viability was observed. Specifically, cell viability was reduced by approximately 50% at γ-tocotrienol concentrations of 200 μM, 300 μM, and 100 μM for young, middle-aged, and elderly fibroblasts, respectively. At toxic doses—500 μM for young and middle-aged cells and 150 μM for elderly cells—cell survival dropped sharply, indicating cytotoxicity at higher concentrations [10].

Further experiments explored the protective role of γ-tocotrienol in the context of oxidative stress induced by H_2O_2. Pretreatment with the optimal dose of γ-tocotrienol significantly protected fibroblasts from all age groups against H_2O_2-induced cell loss ($p < 0.05$), with a notably greater protective effect observed in cells from middle-aged and elderly individuals. This suggests an age-dependent response in which older cells, more susceptible to oxidative stress, may benefit more from antioxidant interventions. Interestingly, similar protective effects were observed when γ-tocotrienol was administered after H_2O_2 exposure, suggesting its potential in both preventive and therapeutic contexts. However, when fibroblasts were treated with high doses of γ-tocotrienol—corresponding to the IC_{50} concentrations—no significant protective effect was detected, regardless of whether the treatment occurred before or after H_2O_2 exposure. This reinforces the importance of dosage in therapeutic applications and highlights the biphasic nature of γ-tocotrienol's action: beneficial at low to moderate doses and harmful at high doses.

Overall, these findings underscore the dual role of γ-tocotrienol as both an antioxidant and, at high concentrations, a potential cytotoxin. Its ability to mitigate oxidative stress-induced cell loss, particularly in aging cells, supports its potential as a therapeutic agent for age-related cellular degeneration. However, careful dose optimization is essential to maximize its protective benefits while avoiding adverse effects.

3. Case analysis

3.1. Effects of oxidative stress and γ-tocotrienol on telomere length

To assess the impact of oxidative stress and antioxidant treatment on telomere integrity, fibroblast cells derived from young and elderly individuals were analyzed using Southern blotting. The results revealed that fibroblasts from elderly donors exhibited significantly shorter telomeres compared to those from young individuals, consistent with the well-established correlation between age and telomere shortening. Exposure to H_2O_2 at IC_{50} concentrations further exacerbated telomere loss in all age groups, confirming the telomere-damaging effects of oxidative stress.

Interestingly, pretreatment with γ-tocotrienol at both optimum and IC_{50} doses provided significant protection against H_2O_2-induced telomere shortening in fibroblasts from both young and elderly donors ($p < 0.05$) [11]. Moreover, administering γ-tocotrienol after oxidative exposure also resulted in a statistically significant attenuation of telomere attrition. These findings indicate that γ-tocotrienol exerts a protective effect on telomere integrity, whether applied before or after oxidative insult, and suggest its therapeutic potential in mitigating age-related telomere erosion.

3.2. Effects of γ-tocotrienol on telomerase activity

To further evaluate the mechanisms underlying telomere preservation, telomerase activity was measured using PCR-based techniques in fibroblast cells subjected to different treatments. Baseline telomerase activity was found to be highest in fibroblasts from young individuals and markedly reduced in cells from middle-aged and elderly donors. Treatment with H_2O_2 significantly suppressed

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telomerase activity in all age groups, supporting the notion that oxidative stress not only shortens telomeres but also impairs the telomere maintenance machinery.

Pretreatment with an optimum concentration of γ-tocotrienol effectively protected against the loss of telomerase activity induced by H_2O_2 in all age groups ($p < 0.05$), suggesting a regulatory role of γ-tocotrienol in telomerase preservation. However, neither pretreatment with high concentrations (IC_{50} dose) of γ-tocotrienol nor post-treatment after H_2O_2 exposure conferred significant protection. This pattern reinforces the dose-dependent nature of γ-tocotrienol's bioactivity and highlights the necessity of precise dosage control in therapeutic applications targeting telomerase activity [12].

4. Nutrition and telomere length

Telomere length varies across human tissues and between species, but in healthy cells, it generally remains within a stable physiological range. Increasing evidence suggests that nutrition plays a significant role in telomere dynamics. Certain dietary components, such as omega-3 fatty acids, polyphenols, and vitamins C and E, have been associated with longer telomere lengths, presumably due to their antioxidant and anti-inflammatory properties.

Collagen, a structural protein with a wide range of biological functions, has recently been implicated in telomere biology. It contributes to skin elasticity, tendon strength, wound healing, and bone health, but also exhibits antioxidant properties by enhancing antioxidant enzyme activity and scavenging reactive oxygen species. Moreover, collagen peptides have demonstrated anti-inflammatory effects by inhibiting the expression of cytokines and enzymes involved in inflammatory responses, such as TNF-α, IL-1β, IL-6, IL-8, iNOS, and COX-2.

A large-scale study using data from the NHANES measured leukocyte telomere length (LTL) via quantitative PCR, and the results indicated a gender-specific association between collagen intake and telomere length. In females, higher collagen intake was positively associated with longer telomeres, while no significant association was observed in males or the general population. Interestingly, the protective effect in women exhibited a nonlinear trend, with the strongest benefit observed at an intake level of 2.5 g/1000 kcal (P for non-linearity < 0.001). Compared to collagen, γ-tocotrienol appears to offer a broader protective range against oxidative stress, without significant gender-based differences in effectiveness, suggesting its advantage in age-related telomere preservation strategies.

5. CRISPR-Cas system and telomere regulation

5.1. Overview of the CRISPR-Cas system

The CRISPR-Cas system, originally discovered as part of the prokaryotic immune defense, has revolutionized genetic engineering. It consists of clustered regularly interspaced short palindromic repeats (CRISPR) and associated Cas proteins, notably Cas9. In bacteria and archaea, the system captures DNA fragments from invading bacteriophages and incorporates them into the host genome as a form of acquired immunity. Upon subsequent infections, these sequences guide Cas proteins to identify and degrade matching foreign DNA, providing efficient and heritable protection.

Cas9, the most commonly used effector protein in gene editing, acts as a programmable endonuclease. Guided by the sgRNA, Cas9 binds to and cleaves specific DNA sequences complementary to the guide. This programmable precision has enabled CRISPR-Cas9 to become a powerful tool for genome editing, with applications ranging from basic research to biotechnology and clinical therapies.

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5.2. Application of CRISPR-Cas in telomere and telomerase modulation

In the context of telomere biology, CRISPR-Cas9 technology presents a promising strategy for manipulating telomere-related genes, particularly TERT and TERC, which encode the reverse transcriptase and RNA template components of telomerase, respectively. While TERC is constitutively expressed in most cells, TERT is usually repressed in somatic cells but becomes reactivated in approximately 80–90% of cancer cells, granting them replicative immortality [13]. Because high telomerase activity is a hallmark of many cancers, TERT has become a prime target for cancer therapies.

Experimental results using quantitative RT-PCR have shown that TERT expression is absent in normal somatic cells such as human aortic smooth muscle cells (HASMCs), but present in cancer cell lines and induced pluripotent stem cells (iPSCs). Correspondingly, relative telomere length measured by the T/S ratio is longest in iPSCs, moderate in HASMCs, and shortest in cancer cells, confirming that telomerase reactivation in cancer occurs independently of telomere length restoration.

The CRISPR-Cas system can be harnessed to silence or disrupt TERT expression in cancer cells, potentially halting their uncontrolled proliferation. Alternatively, precise editing of TERT regulatory elements in somatic cells may provide a means to transiently restore telomerase activity and delay aging-associated telomere attrition without increasing cancer risk. Thus, CRISPR-Cas-based manipulation of telomere maintenance pathways holds great promise both in regenerative medicine and in targeted anti-cancer therapies.

5.3. CRISPR-Cas9-mediated TERT disruption and functional imaging of telomerase

To explore the functional consequences of gene editing on telomerase activity, a CRISPR-Cas9-mediated gene disruption strategy targeting the human TERT gene was employed. This approach involved the design of three single-guide RNAs (gRNAs) aimed at exons 2, 4, and 6 of the TERT gene. Given the central role of TERT in telomerase function and its reactivation in most cancer cells, TERT was selected as a universal therapeutic target for a range of cancer types. To assess the efficiency and generalizability of this editing strategy, the gRNAs were transfected into three distinct human cancer cell lines: HeLa (cervical cancer), PANC1 (pancreatic cancer), and SUM159 (breast cancer). Preliminary data from these experiments suggest that CRISPR-mediated TERT disruption could serve as a broadly applicable anticancer approach by impairing telomerase-driven immortality across different tumor types [14].

In addition to gene disruption, CRISPR-based systems can also facilitate the live-cell imaging of telomerase dynamics. While it is technically feasible to image the genomic region of TERT using catalytically inactive Cas9 (dCas9), such imaging may not fully capture the biological functionality, which primarily depends on the protein activity of telomerase rather than its DNA sequence alone. Therefore, more informative strategies involve directly labeling the telomerase protein or its subunits.

One such study by Schmidt, Zaug, and Cech introduced a fluorescent marker at the TERT locus to visualize telomerase trafficking and interaction with telomeres in live cells. Their observations revealed that telomerase activity can be functionally divided into three distinct stages: a rapid diffusion phase where telomerase molecules move freely within the nucleus, a frequent but transient phase of telomere association, and a rarer, long-lasting binding phase that is primarily responsible for telomere elongation. These stages not only provide insights into the regulation of telomerase but

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also suggest that the timing and frequency of telomerase-telomere interactions are critical for effective telomere maintenance.

Together, these findings reinforce the value of CRISPR-Cas9 not only as a tool for gene disruption but also as a platform for functional genomic imaging. Targeting TERT through gene editing or live-cell imaging opens new possibilities in both telomere biology research and cancer therapy, enabling precise intervention in telomerase-dependent mechanisms of cellular longevity and tumor progression.

6. Conclusion

Telomere shortening plays a pivotal role in the biological aging process and the development of age-related diseases. This review explored the molecular mechanisms behind telomere attrition and evaluated two promising strategies for telomere preservation: antioxidant intervention and gene editing via the CRISPR-Cas system. Experimental evidence demonstrates that γ-tocotrienol, a potent antioxidant, can significantly protect fibroblast cells from oxidative stress-induced telomere shortening and telomerase activity loss, especially in middle-aged and elderly cells. However, its protective effects are highly dose-dependent, with cytotoxicity observed at higher concentrations. Nutritional factors such as collagen also appear to influence telomere dynamics, particularly in women, suggesting a role for gender-specific dietary interventions in aging prevention.

On the genetic level, CRISPR-Cas9-mediated targeting of the TERT gene has shown potential in both inhibiting telomerase activity in cancer cells and enabling real-time imaging of telomerase behavior. These applications offer new insights into telomere regulation and open avenues for therapeutic manipulation. Future research should focus on optimizing delivery systems for gene editing, refining dosage windows for antioxidant compounds, and assessing long-term safety. Together, these strategies may converge to provide effective tools for delaying cellular aging and combating telomere-related disorders.

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