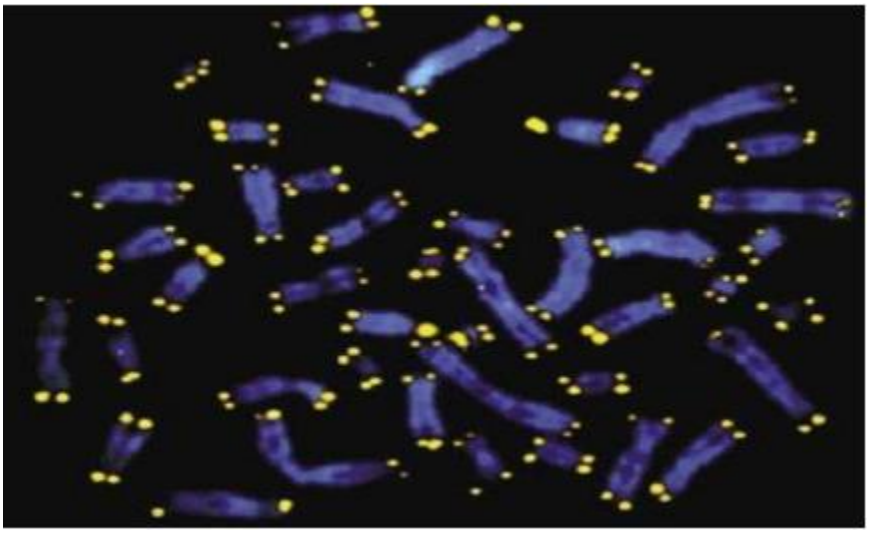
Role of Telomeres in Aging

December 10, 2016 HoJun Kweon and HyoJu Kweon2016 Issue, Biology, Chemistry, Featured, Winter 2016 Issue



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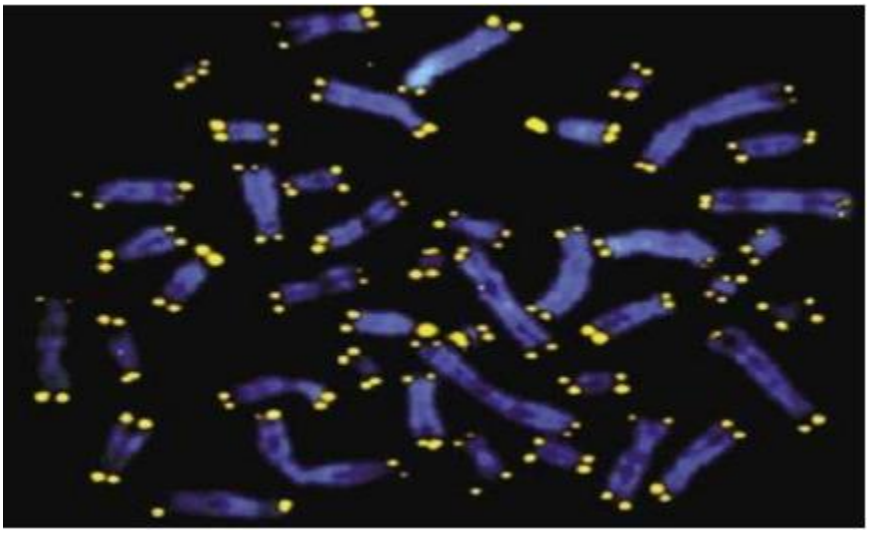


Abstract

At the ends of eukaryotic chromosomes are telomeres, which play a central role in the protection of genetic data and the enabling of cell division. Telomeres modify the cellular response to growth and stress based on various hereditary and environmental factors including, but are not limited to, a person's exposure to oxidative damage, choice of lifestyle, and telomere length. However, as the aging process is influenced by its complex nature and the wide array of genetic diversity among individuals, the comprehensive discernment of age-related factors is highly limited. Furthermore, as cells divide, telomeres shorten and often, in the case of cancers, remain unaffected. Thus, while the activation of telomerase propels tumor cells to malignant states, the subsequent lack of telomerase could contribute to the induction of cancer. In the genetic balancing act between the risks and benefits of either short or long telomere lengths, the molecular mechanisms and implications behind cellular aging are many. Nevertheless, with an increasing number of studies being done on telomeres, telomere biology is becoming a field of greater interest in the scientific community.

Introduction

At the ends of eukaryotic chromosomes are stretches of long, noncoding sequences of DNA called telomeres1. Comprised of repetitive, hexameric sequences of TTAGGG, telomeres serve to protect the loss of genetic data1,2. With telomeres capping the ends of chromosomes, cells divide without the risk of losing genes. Without telomeres, the twisted double-strands of chromosomes fray and fuse together, interfering with the genetic blueprints of cells. Nevertheless, as cells divide, telomeres shorten3.

For a cell to divide and replicate, its DNA double helix must first unwind; as the strands begin to unzip and separate, an enzyme called DNA polymerase "reads" the existing parent strands to construct new daughter strands4. Under the guidance of short RNA pieces called primers, the polymerase assembles nucleotides backwards, in the opposite direction of DNA replication. As DNA is replicated only in the 5' to 3' orientation, the daughter strand is built piece-by-piece with short segments called Okazaki fragments5. As a result, RNA primers are not able to fill in for ends of chromosomes' strands, causing telomeres to shorten with each cell division2 (Figure 1). Thus, telomeres can be seen as a type of cellular timekeeper that limits a cell's proliferative ability3. The continuous shortening of telomeres may lead to senescence, mutations, and apoptosis-termination of cell divisions, the alteration of DNA sequences, and the programming of cell death, respectively.

Telomere shortening, however, can be enzymatically counterbalanced by telomerase, a ribonucleoprotein polymerase that adds a telomere repeat sequence TTAGGG to the 3' end of telomeres. Telomerase, also called terminal transferase, is abundant and active in normal stem cells, but it exists at very low levels in most somatic cells. A telomerase reverse transcriptase (TERT), which mitigates the effects of rapid cellular aging, employs the RNA template of TERC, a telomerase RNA component that serves as a template for the telomere repeat such as CCCUAA, to add telomeric repeats to the ends of chromosomes1. Even so, as cells can only divide a finite number of times, telomere lengths inevitably shorten. On the contrary, telomerase-dependent telomere elongation remains unaffected and active in embryonic stem cells, which divide indefinitely to continue development, and cells of the immune system, which protect against diseases3. Hence, telomere biology research has attracted a great interest in

the scientific community and plenty of researches have been performed in order to find a connection between telomere and cellular aging and a role of telomere in aging.

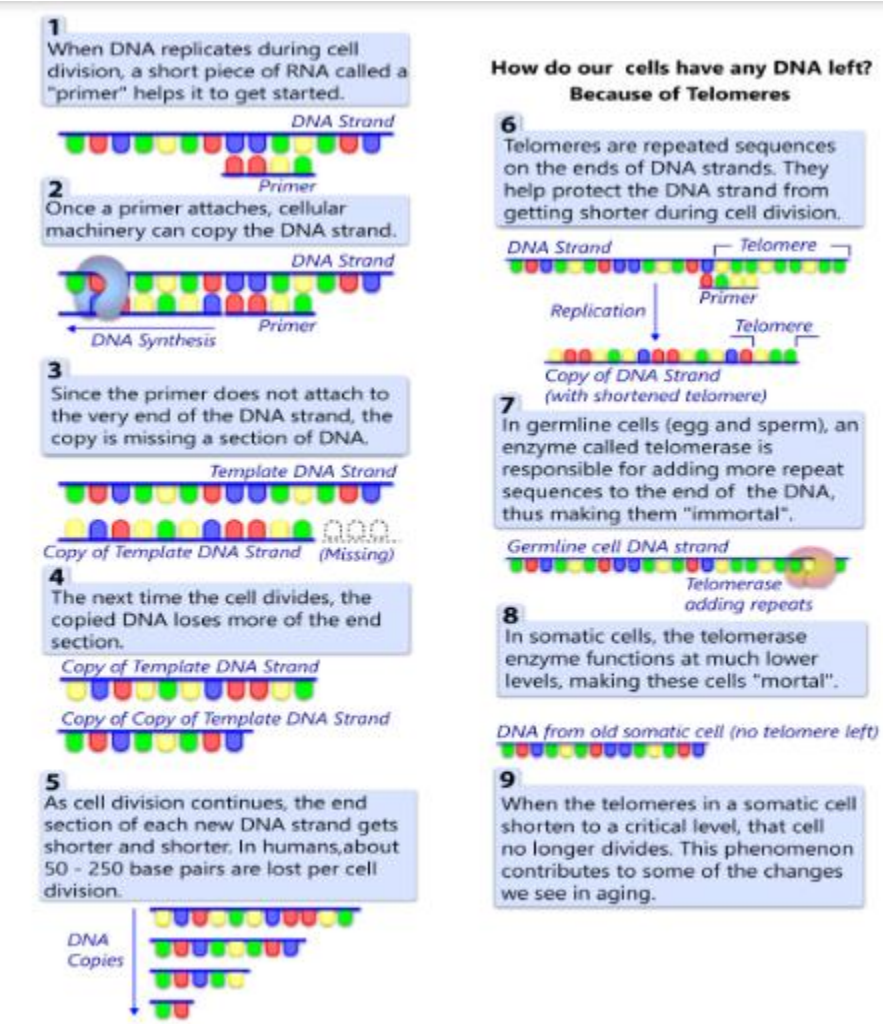


Figure 1. An illustration of telomere shortening.

Telomere and Telomerase Relation to Aging

In 1961, Leonard Hayflick became the first to report that cells divide a finite number of times, a phenomenon now called the “Hayflick Limit.” In 1986, Howard Cooke and his colleagues observed that telomeres in adult somatic cells were considerably shorter than those in haploid germline cells (egg and sperm). Then, in 1990, two scientists, Greider and Carley, put the telomere hypothesis to test and discovered that telomeres did indeed shorten with each cell division. The discovery that cells reach their Hayflick limit when their telomeres shorten to critical lengths has prompted scientists to investigate the molecular mechanisms behind aging6.

Telomere dysfunction signals a cascade of events that leads to cellular senescence and apoptosis, the two major facilitators of tumor suppression. An integral part of dysfunctional telomeres includes the disruption of telomere-binding proteins that

maintain telomere length. Such disruption triggers the activation of p53, which is a tumor suppressor gene, and the p53/p21 senescence pathway7. DNA-damaging events activate the p53 tumor suppressor gene, which in turn prompts the expression of p21, the protein responsible for growth arrest8. Other studies also illustrated possible alternative phenotypic effects of p53-mediated-senescence related to inflammation. In short, telomere dysfunction, caused by telomere shortening, leads to p53/p21-induced-senescence and inflammation, which subsequently leads to tissue damage9.

The dynamics of telomere-independent telomerase may hold our next clue to the secret of aging. Aside from its telomeric function of adding a telomere repeat sequence to the 3' end of telomeres, telomerase maintains the proliferative abilities of cells by governing the expression of mitogenic genes. As such, telomerase overexpression accompanies an increased rate of cell proliferation, and collaterally, telomerase may up-regulate the expression of fibroblast growth factors (FGF) and protect some tumor cells from cell death. Furthermore, telomerase has been shown to transfer apoptosis-resistance mechanisms to various cell types. For example, hTERT, a catalytic subunit of telomerase in human, is known to reduce apoptotic susceptibility. Another function of non-telomeric telomerase is the preservation of mitochondria and mitochondrial DNA (mtDNA) from oxidative damage or reactive substances containing oxygen. The mitochondrial theory of aging contends that an accumulated amount of oxidative damage induces the build-up of reactive oxygen species (ROS), ultimately leading to cell and tissue damage. However, recent studies have suggested that telomerase may be at the center of mitochondrial homeostasis. Telomerase implements regulatory functions within the mitochondria that include a curtailed mitochondrial-derived ROS output, a strengthened mitochondrial membrane, and an enhanced respiratory tract. Collectively, the implications of telomerase activity in cellular proliferation, apoptosis resistance, and mitochondrial function suggest a strong link between telomerase and the aging process9.

Defects in linear telomeric chromosome lead to various diseases. Telomere-induced disease is usually discussed in the context of dyskeratosis congenita, a rare bone marrow disorder that causes premature aging. The disorder is caused by a mutation in dyskeratosis congenita 1 (DKC1), a gene that codes for dyskerin. Unlike progeroid syndromes, disorders that mimic physiological aging, dyskeratosis congenita exhibits signs of progressive aging10. However, as dyskerin is a telomerase-binding protein that stabilizes the telomere, a loss of its function results in markedly reduced telomere length3. Thus, patients with dyskeratosis face an increased risk of complications, including infections, pulmonary complications, and leukemia2.

Other Aging Factors

Aging refers to the deterioration of an organism’s function and body due to changes at the molecular and cellular level. These alterations occur as a result of both genetic and epigenetic factors. However, as the aging process is influenced by its complex nature and the wide array of genetic diversity among individuals, the comprehensive discernment of age-related factors is highly limited11.

As mentioned, telomeres are the timekeepers of cells. Research led by Dr. Cawthon and his colleagues gave further credence to the idea that shorter telomeres correlate with shorter lives. Among 143 participants aged 60 and above, individuals with shorter telomeres exhibited a threefold higher risk for heart diseases and an eightfold higher

risk for other infectious diseases12. However, telomere length alone is not enough to determine lifespan.

One of the leading theories in the mechanism of aging is “oxidative stress,” the impairment of DNA, proteins, and lipids13. Reactive oxygen species (ROS), highly reactive and unstable chemical species such as superoxide anion (O2?), hydroxyl radical (•OH), and hydrogen peroxide (H2O2), cause damage in several ways: by readily attacking DNA, which causes chromosomal instability, by inducing telomere shortening, which leads to cellular senescence, and by interfering with the mitochondria, which induces apoptosis. Mitochondria are the main producer of ROS and are thought to be the primary target of oxidative damage that occurs during oxidative phosphorylation of mitochondrial macromolecules. The indication of mitochondrial dysfunction in aging could be explained by increased prevalence of mitochondrial DNA (mtDNA) mutations/deletions and decreased mtDNA abundance. Despite such evidences, the positive link between oxidative damage and aging remains elusive and recent studies have begun to shed more light on this matter. An increased output of ROS causes cellular damage to result in aging and furthermore, the low level of ROS under normal conditions is found to act as signaling molecules in many necessary physiological processes. These results suggest that ROS can be detrimental byproducts and also important mediators for a variety of signaling pathways depending on the levels of ROS, which could be balanced by various antioxidants14.

What we eat, how often we exercise, and how we manage stress all hold secrets to why and how we age15. Intuition has it that a healthy and abstemious lifestyle accompanies longevity. In fact, a five-year pilot study conducted by scientists at UC San Francisco supports this idea. Blood samples from 35 men with early prostate cancer found an association between shorter telomeres and mortality. Ten of the participants in the experimental group were asked to undergo drastic changes to their lifestyles that consisted of a whole food-based diet, a daily exercise regime, an organized means of stress reduction, such as yoga, and increased social interaction. In contrast, the remaining 25 men in the control group were asked to adhere to their original lifestyles. By the end of the five-year study, the experimental group had measurably longer telomere lengths by ten percent, while the control group ended with shorter telomeres of approximately three percent. Researchers noted that the relevance of the new research should extend beyond prostate cancer patients to the general population (Figure 2)16.

When telomere length, choice of lifestyle, and chronological age are combined, the mechanism to getting cancer may be uncovered. Just as telomeres have been noted as a type of biological clock, they have also been proposed as a biomarker of cancer17. In somatic cells, telomeres undergo repeated shortening with each cell proliferation. On the contrary, in cancer cells, telomeres undergo unlimited cell replication15 and thus telomerase is reactivated, enabling cells to become virtually immortal. Short telomeres alone lead to chromosomal instability, which may lead to cancer initiation and development18. Here, we see two mechanisms at odds with each other. While the activation of telomerase propels tumor cells to malignant states, the subsequent lack of telomerase could contribute to the induction of cancer.

Figure 2. A USCF pilot study found that lifestyle changes may lengthen telomeres, which are associated with healthy behaviors.

Current Research

That telomeres are transcriptionally silent is no longer a credible statement. In fact, Dr. Lu and his team at Zhejiang University have discovered that telomeres are transcribed into telomeric repeat-containing RNA (TERRA), long non-coding RNA that regulates telomere length19. TERRA stimulates telomere shortening in several ways. Firstly, the mutation of Rat1p, a yeast RNA exonuclease, results in an excess of TERRA, forming DNA/RNA hybrids that inhibit telomerase activity, and consequently, telomere repair. Secondly, TERRA promotes the activity of exonuclease-1 (Exo-1) by interacting with the Exo1-inhibiting Ku70/80 dimer, which facilitates the resection of chromosome ends to initiate telomere shortening. In biological system, protein Exo-1 is involved in DNA mismatch repair and homologous recombination (HR) and Ku70/80 dimer, a protein that binds to DNA double-strand break (DSB) ends, is required for the non-homologous end joining (NHEJ) pathway of DNA repair and telomeric length maintenance20. Thirdly, TERRA increases the production of euchromatin and

decreases the production of heterochromatin to adversely affect telomere length. Consisting 92% of the human genome, euchromatin is a lightly packed form of chromatin composed of DNA, RNA and protein. On the other hand, heterochromatin is a tightly packed form of DNA, which forms structural functions such as centromeres or telomeres. The increase of heterochromatin is essential for telomeric length maintenance.

Furthermore, though TERRA serves to preserve chromosome ends, this RNA may actually pose more damage than protection. Telomeric repeat-binding factor 2 (TRF2) is a component of telomere-shelterin complex and has been observed at the loop-tail junctions of telomere loops (T-loops). As shown in Figure 3, a 300 bp single-stranded portion at the very end of the telomere forms the large telomere loop, T-Loop21, which stabilizes the telomere and prevents the telomere ends from being recognized as break points by the DNA repair protein. This T-loop contains several proteins, such as TRF1, TRF2, POT1, TIN1, and TIN2, altogether called as the shelterin complex. The formation of T-loop is promoted by TRF2 by the invasion of the 3' telomeric overhangs into the duplex telomeric array. These T-loops act as a protective cap to prevent DNA damage. At the very end of the T-loop, the single-stranded telomere DNA forms another triple-stranded structure, called a displacement loop or D-loop.

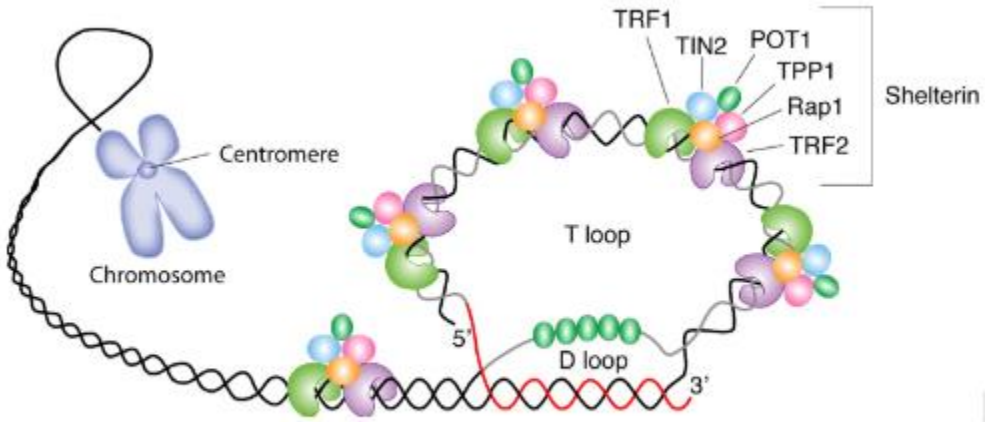


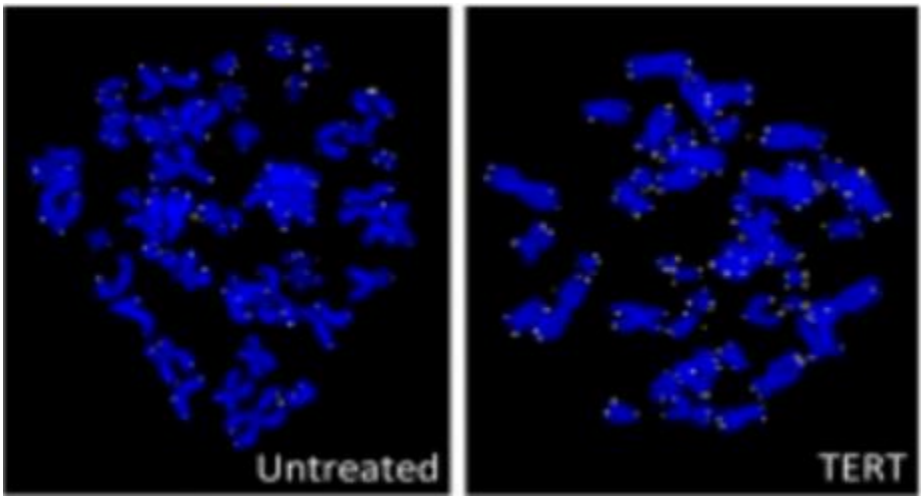
Figure 3. Schematic representation of telomere structure.

It has been further noted that it is the T-loop deletion caused by homologous recombination (HR), which causes the exchange of nucleotide sequences between similar or identical chromosomes, to accelerate telomere shortening. TRF2 that promotes T-loop formation is found to suppress T-loop HR by inhibiting branch migration and/or strand cleavage. Thus, the activation of T-loop HR, which is induced by TERRA binding to TRF2 by forming an intramolecular G-quadruplex structure, promotes the rapid shortening of telomeric DNA19. Thus, telomere shortening can be prevented by inducing telomere elongation by both telomerase and the telomere maintenance mechanism (TMM), which depends on homology-directed repair (HDR). Overall, TERRA has an important role in regulating telomeric length for the protection of chromosome ends by maintaining the dynamic balance of its levels.

Currently, the most utilized method of telomere extension is the delivery of viral TERT to human cells. Recently, however, Dr. Blau and her colleagues at Stanford University

conducted a delivery of TERT by non-viral, non-integrative methods22. Though transient in effect (24-48 hours), the delivery of modified mRNA encoding TERT to human fibroblasts and myoblasts rapidly extended telomere lengths. Throughout the experiment, cell proliferative capacity increased in treated human fibroblast cells in a TERT mRNA dose-dependent manner, whereas the number of untreated human fibroblast did not change after 50-60 population doubling (PDs) (Figure 4). In a similar manner, treated human myoblasts exhibit a ten-fold increase from the average cell number of untreated myoblasts. According to the study, although the effect of modified TERT mRNA treatment is transient, the treatment of modified mRNA encoding TERT to both human fibroblasts and myoblasts efficaciously extends telomere lengths by increasing telomerase activity. Because of the ability to delay senescence and increase cell proliferative capacity without the risk of cell mutagenesis, such method holds much merit in the scientific community. Therefore, this method constitutes a potential that will lead to breakthroughs in the biological research and medicine of diseases22.

Though longer telomeres are generally thought to confer health and longevity, researches at UC San Francisco have revealed otherwise23. Longer telomeres may be associated with fatal brain cancers called gliomas. During the first stage of the genomic study, researchers drew data from a pool of 7,736 healthy individuals and 1,644 glioma patients. Here, they identified telomeric variants, TERT and TERC, as glioma risk factors. Then, in the second part of the study, researchers analyzed additional data from 40,000 individuals and confirmed that TERT and TERC were not only associated with glioma but also with longer telomere lengths. However, their findings further revealed that short telomeres afford a contradictory phenomenon- a higher risk of cardiovascular disease. Overall, the analysis of a large genomic dataset suggests that, “both longer and shorter telomere length may be pathogenic, depending on the disease under consideration.”



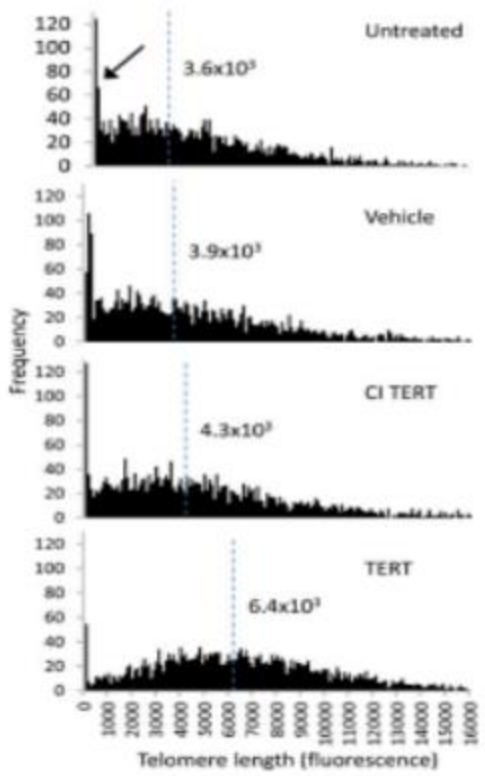


Figure 4. Telomere length analysis of fibroblasts treated with TERT mRNA. Top: yellow represents the signal from the telomere probe and blue is DAPI, a fluorescent stain. Bottom: frequency distributions of telomere signal intensities represent telomere lengths for each population where vertical dashed blue lines indicate median telomere signal and arrow indicates the distribution of the corresponding shorter telomeres. CI TERT: catalytically inactive TERT.

Prospective

There are plenty of researches that both support and oppose the link between telomere and aging, but to date, the dichotomy remains unresolved. Do short telomeres merely exhibit signs of aging or actually contribute to the aging process? However, one aspect remains certain: the merit that telomere research holds in the scientific community.

Over the past decade, much emphasis has been placed on studies targeted at telomeres and telomerase. As aforementioned, TERRA plays a critical role in telomere maintenance. The RNA can either promote telomere shortening by inhibiting telomerase activity or induce the rapid condensation of telomeric DNA by binding to TRF2. Excitingly, however, TERRA may provide progress in the field of cell reproduction, as scientists were able to detect increased levels of TERRA not only in fetal oocytes but also in spermatogenesis19.

Furthermore, the new approach of delivering TERT by non-viral, non-integrative methods holds potential uses for therapy in treating both aging and genetic related

diseases. Dr. Blau's findings on the positive link between the muscular stem cells of patients with Duchenne muscular dystrophy and shorter telomeres hold implications for understanding the role of telomeres in muscle cell formation. Whether or not cells actually function in the making of muscle cells, the study is a step towards advancing cell therapies. Perhaps it may become possible to treat Duchenne muscular dystrophy one day by effectively extending patients' telomeres lengths22.

Untangling the relationships between diseases and telomere lengths has proven a difficult task. Whereas longer telomeres confer signs of health, they also cause cancer cells to reach malignant states. Thus, many applications are now being explored in clinical trials, with many drug companies seeking to block telomerase activity in tumors. Furthermore, Walsh and his colleagues at UC San Francisco have contended that their findings not only pertain to gliomas but also to lung, prostate, testicular and breast cancers, where TERT variants have been implicated, and leukemia, colon cancer and multiple myeloma, where TERC variant levels are high23.

Conclusions

There are still controversies on whether aging is caused by telomere shortening or whether aging can be reversed by telomere elongation. Furthermore, how telomeres are mechanistically involved in age-related diseases and how telomeres and telomerase can be effective targets for the treatment of telomeropathies remain uncertain. However, we cautiously conclude that a healthy human lifespan can be maintained based on a clinical balance between the benefits and risks of telomere lengths: not too short or long, but intermediate. With ongoing research and reports on meaningful scientific data, we believe that there is still a lot to learn about telomere biology.

Abbreviations

TERT: Telomerase reverse transcriptase; TERC: Telomerase RNA component; FGF: fibroblast growth factors; mtDNA: mitochondrial DNA; ROS: reactive oxygen species; DKC1: dyskeratosis congenita 1; TERRA: telomeric repeat-containing RNA; HR: homologous recombination; PDs: population doublings; TRF2: telomere repeat binding factor-2; DAPI: 4',6-diamidino-2-phenylindole (a fluorescent stain).

Acknowledgements

We would like to thank Dr Kaapjoo Park (kaapjoo@gmail.com) for his excellent mentorship and guidance in the preparation of this paper.

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