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*REVIEW ARTICLE*

# THE ROLE OF TELOMERIC ACTIVITY AND TELOMERASES IN AGING WITH NEOPLASIC CHANGES

Esra BİLİCİ[1*]

[1] Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Medical Biology and Genetics, Afyon, vet.hekimesrabilici@gmail.com, ORCİD:0000-0001-6636-5975

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## ABSTRACT

The effect of telomeres on aging and cancer is very important. Telomere is a necessary structure for the continuous preliferation of human cells and is vital for most cancer cells. Telomeric structures located at the ends of the chromosomes consist of TTAGGG repeat units. Telomere terminal transferase is the enzyme responsible for telomere synthesis. It is also a large enzyme complex. Reverse transcriptase provides activation by strengthening the wearing parts after high telomere loss. In addition, it has been suggested that there are cancer cells that do not have telomerase activity but are able to extend the length of telomeres. In the timeframe of cellular division, telomerase enzyme can repair these errors if telomere sequences are lost. In cases where it is not repaired, the protection of these areas is eliminated. Thus, shortening occurs at the ends of the chromosomes. It has been researched by scientists that this shortening causes cellular aging. Reverse transcriptase enzyme has been reported to cause tumorigenic transformation of human epithelial cells and fibroblasts by cooperating with a number of oncogenes and suppressing several tumor suppressor genes. Studies on telomere shortening prove that this enzyme can have a strong effect in the treatment of cancer and is an important development for many patients who are expecting hope. Studies conducted in recent years are among the ideas that the structures and telomerase activity of telomere regions play an active role in cell aging and cancer formation. In the light of all these data, there is not a complete solution of aging, but studies are still ongoing today and major steps have been taken regarding cancer treatment. In this review, the definition of telomeres, their purpose, measurement methods and current studies are given.

**Keywords**: *Telomere, Telomerase, Cancer, Aging*

## 1. INTRODUCTION

Hermann J. Muller described the expression of telomere for the first time by studying the Drosophila melanogaster chromosome in 1938 and examined the structural changes that occur after X radiation and the frequency of these changes. As a result of these examinations, it was observed that deletions and inversions occurring at the ends of the chromosomes were less common. In further studies, it has been observed that the broken-ended chromosomes coalesce easily and the telomere structures of normal chromosomes are stable. As a result, it has been accepted that there are special terminal structures that allow chromosomes to form integrity [1].

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Telomeres are nucleoprotein structures that prevent the ends of chromosomes from being recognized as DNA breakage and function to maintain genome stability [2,3,4]. Mammalian telomeres are composed of repeats of the TTAGGG DNA sequence linked by a six protein complex called shelterin. Due to the recent replication problem [5] telomeres shorten with each cell division, leading to progressive telomere wear that is considered one of the mechanisms underlying organmal aging [6,7]. When telomeres are critically shortened, they trigger a persistent DNA damage response in cellular aging or apoptosis at chromosome ends [8], ultimately compromising the regenerative capacity of tissues [9].

Telomeres are heterochromatic areas that are located at the ends of chromosomes of eukaryotic organisms and consist of specialized DNA repeat sequences [10,11]. It consists of subtelomeric region and main telomere DNA. The main telomeric region is 10-15 kb in length in humans [12] and is the continuation of the subtelomere. The main telomere region consists of telomere DNA consisting of repetitive sequences and telomere-bound structural proteins. The feature that separates telomeres from the rest of the chromosome; It is the loss and regaining of telomeric DNA due to the cell cycle, which is called 'telomere dynamics'. In human somatic cells, telomere dynamics proceed negatively. The telomeric DNA lost by the cell in each cycle is more than the telomeric DNA to be synthesized again [13].

Thirty years ago, the classic view was that telomeres retain the natural ends of linear chromosomes and that telomerase is a specific telomere-terminal transferase required for the replication of chromosome ends in unicellular organisms. Although this concept is still valid today, many different areas related to telomeres and telomerase have matured significantly. These areas include the discovery of many of the key molecular components of telomerase, the limitations of cellular replication, the identification and characterization of human genetic disorders that cause premature telomere shortening [14].

It is known that telomeres are resistant to exonuclease and ligases, play a role in the stability of chromosomes, but also play a role in nuclear construction and gene expression. In addition, telomeres prevent chromosome ends from sticking to each other by ensuring the completion of replication [15]. Telomeres protect chromosomes from being detected as damaged DNA and from recombination and splicing;ensures full replication, functional organization of chromosomes in meiosis and nucleus; it also acts as a molecular clock to determine a cell's division capacity [16-18].

## 2.STRUCTURE AND SUB-UNITS OF THE TELOMERASE ENZYME

The structure responsible for telomere synthesis is called telomerase (telomere terminal transferase or reverse transcriptase) enzyme [1]. Telomerase is a reverse-transcriptase synthesized from a single strand of telomeric DNA consisting of a combination of RNA and protein. However, it is also a large enzyme complex [19]. Telomerase is a ribonucleoprotein complex containing a catalytic core, telomerase reverse transcriptase (TERT) and non-coding human telomerase RNA (hTR) that serves as a template for the insertion of telomeric repeats at chromosome ends [20]. The telomerase enzyme has three components. These are: Telomerase RNA subunit (TR), Telomerase protein component (TP1), Telomerase catalytic subunit (TERT) [21].

Telomerase RNA subunit (TR) differs from other terminal transferase enzymes in that it uses its own RNA subunit as a template. This subunit is transcribed by RNA polymerase II and is used as a

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template for attachment and reverse transcription to telomere DNA on the side close to its 5-end. For this, it is thought to be suitable for anti-telomerase therapy [22]. The telomerase protein component TP1 is the regulatory component of the telomerase enzyme and binds specifically to the RNA subunit of telomerase. It is thought to be ineffective in controlling telomerase enzyme activity. The catalytic subunit of telomerase (TERT) is the portion of telomerase with reverse transcriptase activity. Cancers, through TERT activation or alternative telomere elongation, require telomere maintenance mechanisms for their unlimited potential for division [23]. Complementary bases are added by TERT in accordance with the mold. The mold part of the TR is attached to the telomere, resulting in long telomere length [24]. TERT is generally similar in all living things, but it is known that there are some differences between them. Telomerase has emerged as an important primary target in anticancer therapy. It is a distinctive reverse transcriptase enzyme that elongates telomere length at the 3 'chromosome end and uses domains containing telomerase reverse transcriptase (TERT) and telomerase RNA template. Telomerase is a contributing factor to human health and has a vital role. It mainly affects cell aging and cell proliferation. Because of its unique feature, it provides unlimited cell proliferation in malignancy and plays a major role in cancer [25]. The complex called reverse-transcriptase in humans has two subunits that are important for telomerase activity: human telomerase RNA (hTER),and human telomerase reverse transcriptase (hTERT) [12,26,27].

The gene encoding "h-TERT", which is the catalytic subunit of telomerase, was cloned in 1997 and is known to contain 7 exons. The first exon is specific to telomerase, while others are similar to those of other reverse transcriptase [28]. The strong link between telomerase activity and the expression level of the gene encoding "h-TERT" suggests that "h-TERT" may be responsible for regulation of telomerase activity [29]. Control of telomerase activation in humans has been recognized only at the level of hTERT, as other components are expressed repeatedly [30]. hTERT is the main mechanism in the regulation of telomerase activity. While it negatively regulates the transcription factors p53, IFNα and TGF-β hTERT, it has been reported to positively regulate Human Papilloma Virus 16E6 and estrogen [30-32].

The human telomerase RNA component (hTER) acts as a template for the synthesis of telomeric repeats with 1-6 5'-CUAACCCUAAC-3 'sequences at the 5' end. The hTER gene is the only duplicate gene located in the 3q26.3 region. Recent studies have shown that hTER can act as an oncogene [33,34].

**2.1. Working Principle of Telomerase Enzyme**

During cell division, an exception occurs at the ends of the linear chromosome in the telomeric region of the chromosome [35]. During cell division, DNA polymerase does not start DNA synthesis directly. Replication occurs from the top 5 to the top 3, but can occur with a pre-RNA fragment of 8-12 bases. This fragment is called "RNA Primer". In order for the DNA polymerase to bind, the RNA primer forms a 3'-OH group. As a result, DNA polymerase; it continues its reading in the batch and continuously synthesized yarn. Since the resulting DNA moves in the 3-way direction, after the RNA is removed, there will be an unfilled portion at the 5-top end of the new double-stranded DNA molecule. This part cannot be cured by DNA polymerase. As a result, the chromosome will theoretically shorten to the length of the RNA primer at the end of each synthesis. Telomerase prevents telomere shortening by attaching to the ends of chromosomes after cell division takes place. Hydrogen bonds are formed between strings curling in the shape of a "hairpin" and mutually lined guanosines. The free 3'-OH end required for DNA polymerase I to fill the gap is formed when the

RNA primer is removed. The hairpin structure is then broken and DNA loss after the cell cycle is prevented [35].

**2.2. Telomerase Measurement Methods**

It is known that there are more than one method to determine telomerase activity. The first known method is TRAP (Telomeric Repeat Amplification Protocol) method, which is the reproduction of telomeric repeats based on PCR. Kim et al started using this method in 1994. After the TRAP method was started to be used, it turned out that this method was not sensitive enough and after it did not give reliable results, the positive and negative results could not be fully evaluated. Determination of telomerase activity in tissues has enabled the investigation of telomerase expression in more than one cancer type with the development of the method. In order to overcome the difficulties faced, TRAP-eze and TRAP-eze-Elisa kits, which are improved versions of this method, have been used [36].

PicoGreen method is a method developed by Gelmini et al. In 1998. PicoGreen used in naming this method is a fluorescent dye and is based on selectively binding to double stranded DNA. "Stretch-PCR" method is used for the growth of telomerase product [37].

The Transcription-Mediated Amplification and Hybridization Protection Assay (TMA / HPA) method was proposed by Hirose et al. (1998). This method is very simple and can be applied quickly. In addition, it is less affected by TRAP inhibitors that can be obtained from clinical data. There is no drawback in using a water bath in the transcription step, since the redunanz is fast with the transcription of the telomerase product. At the same time, since several billion RNAs can be replicated from a template within an hour, the TMA / HPA method is based on different hydrolysis of probes that hybridize with RNAs or not, and the addition of a promoter to the primer to be extended by the reverse transcriptase enzyme [38].

Aldous and Grabill (1997) used the Fluorescent-TRAP method (F-TRAP) to determine the telomerase activity. This method uses fluorescence-labeled primers and the use of radioisotopes is eliminated [39].

As a result of the studies carried out by Zavlaris et al. In 2009, it is thought that if the TRAP (Telomeric Repeat Amplification Protocol) method is to be used, the application of "stretch-PCR" with the PicoGreen method, and the determination of the gene expression of TERT, even if the Real Time PCR method will be used, may be preferred [40].

## 3. NEOPLASIC CHANGES AND TELOMER RELATIONSHIP

It was determined that telomerase activity can be detected mostly in cancer cells and rarely in normal somatic cells. After embryogenesis, in contrast to the loss of telomerase activity in most somatic cells, it has been shown that there is little telomerase activity in somatic cells such as hematopoietic stem cells, skin and intestinal epithelium cells, esophageal epithelium, gamete cells, endometrium cells and hair follicles that can regenerate themselves significantly [ 41]. This suggests that this enzyme is active in cells with high growth rates, whether normal or malignant [42].

Telomerase provides activation after high loss of telomere, by strengthening the worn out ends [43]. It was first suggested by Olovinikov in 1973 that cells lose some DNA from their terminal ends at each replication and this shortening observed in chromosomes leads to cellular aging [44,45]. The shorter

telomere lengths of somatic cells such as fibroblasts and leukocytes in the elderly compared to young ones also supports this hypothesis. In addition, it has been suggested that there are cancer cells that do not have telomerase activity but are able to increase the length of telomeres. It has been reported that this enzyme cooperates with a number of oncogenes and causes the tumorigenic transformation of human epithelial cells and fibroblasts by suppressing several tumor suppressor genes. In the light of this information, it is thought that telomerase may play a role in cancer diagnosis and follow-up, so that telomerase activity may lead new approaches in the fight against cancer [46].

Telomerase, a key enzyme for cell survival, prevents telomere shortening and cellular aging observed after many cells divide. In contrast, telomerase inactivation is observed in most cells of the adult liver. Lack of telomerase activity and shortening of telomeres have played a role in hepatocyte aging and hepatocellular carcinoma (HCC) development. Telomerase reactivation is required to induce uncontrolled cell proliferation leading to HCC development [47].
Telomerase activity yields eukaryotic cells with unlimited proliferation capacity, one of the hallmarks of cancer. More than 90% of human urothelial carcinoma of bladder (UCB) tumors are positive for telomerase activity. Telomerase activation can occur by several mechanisms. While mutations in the core promoter region of the human telomerase reverse transcriptase gene (TERT) cause telomerase reactivation in 60-80% of UCBs, the prevalence of these mutations is lower in urothelial cancers of other mutations. In the future, TERT promoter mutations and telomerase activity may have diagnostic and therapeutic applications in UCB [48].

Prostate cancers protect telomeres predominantly by activating them, but in metastatic disease alternative mechanisms of telomere elongation may occur. Telomerase activity and telomere length assessment may be helpful in prostate cancer diagnosis and prognosis. Disruption of androgen receptor function in prostate cancer cells leads to telomere dysfunction, indicating telomeres and telomerase as potential therapeutic targets in prostate cancer. While telomere shortening in normal stromal cells has been associated with prostate cancer, variable telomere lengths in prostate cancer cells and telomere shortening in cancer-related stromal cells have been associated with lethal disease [49].

Chondrosarcomas are malignant skeletal tumors with chondroid differentiation. The prognosis is largely dependent on histolopathologic grading as double-blinded by two scientists. Telomerase activity and abundant expression of telomerase reverse transcriptase (hTERT) have previously been associated with chondrosarcoma grade and metastasis. Studies have shown that hTERT promoter mutations are common in high-grade conventional chondrosarcomas and strengthen the rationale for telomerase targeted therapy in a chondrosarcoma subset [50].

Alternative telomere extension mechanisms are generally absent in meningiomas. TERT and hTERT promoter changes play an important role in prognosis and potentially treatment during oncogenesis of meningiomas [51].

Recently, the G-quadruplex (G4-DNA) structure found in telomeres has been discovered. These nucleic acid sequences, also called G-tetrad, are rich in guanine. Composed of guanine, G-quadruplex refers to a formation containing four chains. Compounds that stop telomerase activity and provide stable structure of G-4 DNA at the same time prevent telomerase enzyme from reaching telomere and stop telomerase activity have started to attract attention in cancer treatment. These compounds

preserve the G4-quadruplex structure and prevent the reverse transcriptase enzyme from reaching the telomeres [52].

DNA G-quadrupoles have been potential drug targets for cancer therapy [53–56]. G-quadrupoles with various functions have attracted attention as a supramolecular synthesis tool to create nanomaterials [57]. G-four poles are four-stranded structures formed in guanine-rich arrays held together by the guanine-guanine Hoogsteen hydrogen bond. The formation of G-quaternary structures is common in regions of biological significance such as human telomeres and oncogene promoter regions [58–60]. Human telomeric DNA, consisting of 5-8 kb tandem repeat d (TTAGGG) n sequences and terminating with a 100-200 nt 3′ single-stranded overhang, forms caps by nucleoprotein complexes and plays an important role in cell aging and death [4,61-64 ]. Telomerase has been shown to be active in 80–85% of human cancers to prolong telomere and increase the survival of cancer cells [65,66]. Previous studies in human cancer cells have demonstrated the formation of DNA G-quadrature in telomeres, and G-quadruple stabilization by small molecules has been shown to induce tumor cell aging and apoptosis by suppressing telomerase activity and DNA damage response pathway [67–71]. Therefore, there has been great interest in stabilizing G-quadruplexes as potential drug targets for cancer therapy development and small molecules [72].

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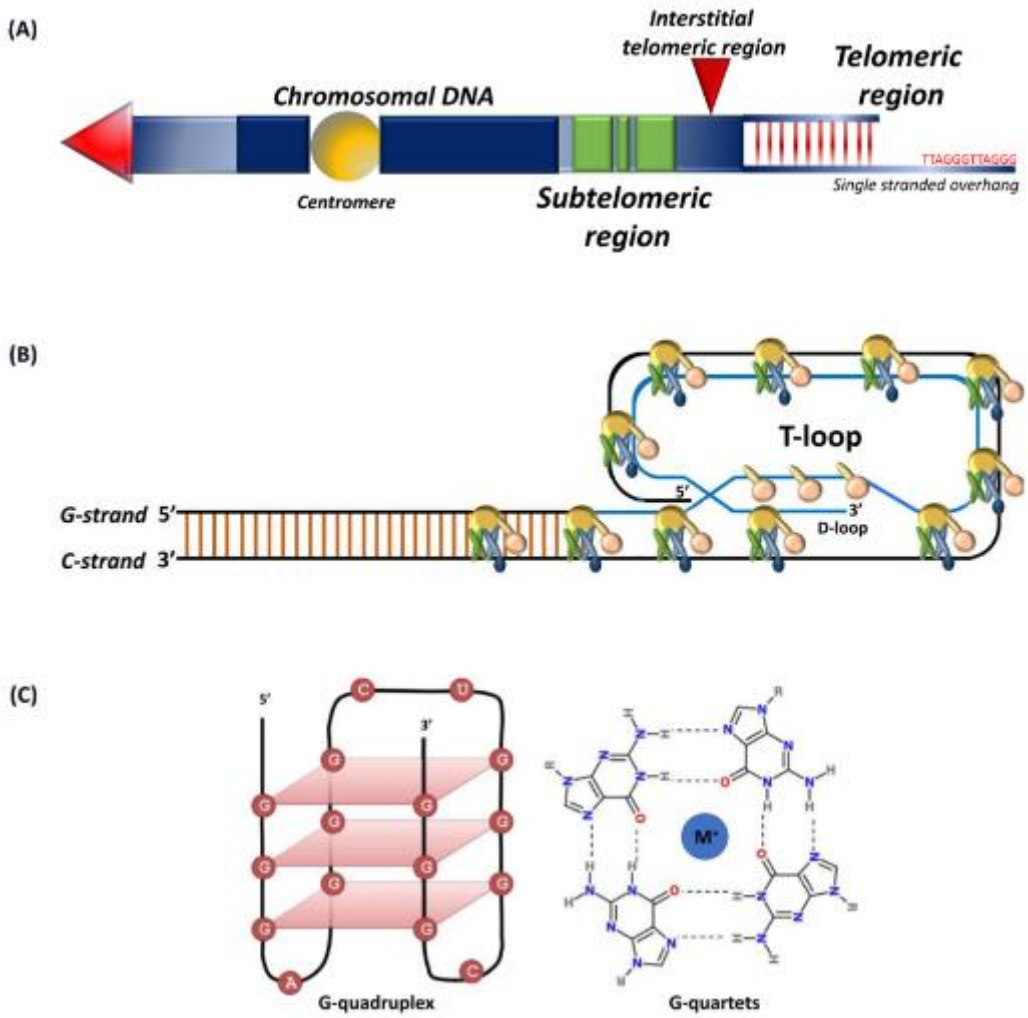


**Figure 1.** (A) Schematic representation of telomeres and lower telomeric regions. (B) The shelterin complex is folded back into duplex DNA to form the G-tail t-ring; (C) G-quartet formed by the cyclic Hoogsten hydrogen bonding arrangement of four guanines with the G-quadruplex structure [73,74].

## 4. AGING AND TELOMER RELATIONSHIP

Aging is a biological process that is characterized by a progressive functional decline in tissues and organs and ultimately leads to death [75]. During cell division, an incomplete copy of the DNA of each chromosome is made, causing the telomeres to shorten in successive generations. When a threshold length is reached, replication stops and the cell "ages" [76]. Telomeres play an important role in cellular aging as they are shortened every time the cell divides and trigger a DNA damage response that results in aging [6,8]. Cells with unlimited proliferation potential need to expand their telomeres to avoid cessation of permanent growth. In humans, telomeric DNA is synthesized by the

telomerase enzyme. This RNA protein complex is active in germline and stem cells, but is absent in most somatic cells [77]. In eukaryotes, telomeres determine cell proliferation potential by triggering replicative aging in the absence of telomerase [78].

Telomerase deficiency leads to progressive telomere erosion in human cell division due to the inherent property of DNA polymerase [14]. When telomeres shorten to a critical size and telomeres become dysfunctional, the DNA damage response pathway is activated and cells begin to enter a permanent growth arrest phase called replicative aging. It is believed that aging is a very effective barrier against cancer by blocking proliferation and genetic mutations that result from DNA replication. Since infinite proliferation is the hallmark of malignant cells [79], it is likely to overcome the aging barrier with telomere stabilization in oncogenesis, and in most cases this is achieved by telomerase activation [14,79].

Aging in adult tissues comes into play in response to different types of damage. One of the movements that cause aging is damage to telomeres, highly repetitive DNA structures located at the end of chromosomes. Telomeres are protected by a multiprotein complex known as shelterin. By coating on telomere, shelterin prevents the activation of a DNA damage response, thus preventing end-to-end chromosome assembly that would lead to telomere crisis [80]. The end-of-replication problem is a consequence of the inability of DNA polymerases to synthesize DNA without a template occurring in telomeres. This results in telomeres gradually shortening with each cell cycle division. Embryonic tissues circumvent this erosion by expressing telomerase, which serves to join DNA to the ends of chromosomes and thus provides a template for DNA synthesis [81]. However, in telomerase-deficient adult tissues, repeated cell division results in progressive DNA erosion, decreased shelterin binding, and aging. As an organism ages, cells accumulate more divisions. This results in increased telomere erosion and aging. However, it is not known to what extent telomere erosion affects aging during aging and to what extent the aging process contributes to it [82].

Aging and death occur in human cells in two stages. The first of these is the replicative aging M1 named as "Mortality Stage". At this stage, chromosomes reach a critical size as a result of shortening of telomeres. Hayflick and Moorhead first described the critical point definition [83]. Leonard Hayflick studied replicative aging and the cells of young people showed the most division in culture medium than cells of the elderly. After dividing about 60 - 80 times, Human embryo cells have started to age. He reported that while senescent cells remained metabolically active, no new cell formation (Hayflick Limit) and eventually died [84]. This event stopped the cell cycle and initiated the senile program [18].

It is the M1 point that represents the replicative life span. If a cell passes the mortality stage, its telomeres shorten to the M2 point. M2 point is called "crisis" or "second mortality stage". Major cell death occurs in this second mortality stage. The cause of cell mortality may be due to chromosome death due to weakened telomere function. Telomerase activity is required to prevent these mortalities. As a result, telomere length and structure can be restored and cells formed at the M2 point can divide infinitely (cellular immortalization). Data obtained as a result of research to date revealed that one of the characteristic features of aging cells is telomeres [85].

Age is a strong prognostic indicator of reduced survival in many cancers [86]. Senescence is a potent tumor suppressor mechanism that limits cancer onset through both cell-intrinsic [87] and cell-extrinsic mechanisms [88]. Aging cells may contribute to tumor progression by increasing the proliferative

potential of cancer cells [89] or by contributing epithelial cells to the mesenchymal transition [90]. Therefore, the increasing number of senescent cells found in aged tissues may contribute to the increase in cancer incidence with age. Supporting this, a delayed onset of tumor formation is observed when senescent cells are eliminated [91].

## 5. CONCLUSION

From the original study of Barbara Mc Clintock [92] in 1941 until today, it is obvious that telomere structures are important in terms of ensuring chromosomal integrity. There are several studies showing that the mechanism that keeps telomere loss in balance in vertebrates is telomerase enzyme. For this reason, reverse transcriptases are of great interest in cancer therapy. Preventing the functionality of telomerase leads to shortening of the extremely important telomeres. As a result, chromosomes that are shorter cause instability and mortality. It is known that telomerase expression is closely related to cellular immortalization and early stages of cellular change. However, it has nothing to do with the growth rate. In other words, we can say that immortal cells regulate telomerase expression. This event demonstrates how important replication is for telomerase activation [93].

The systems responsible for the elongation of telomeres in tissues during division cannot continue their activity. Therefore, telomeres shrink during cell replication. Telomer length determines the replicative survival time of cells. When telomeres reach critical stature, the aging program is activated. Cell replication then stops. But they continue to live and function. Telomeres are actively maintained in reproductive cells because it is imperative that chromosome be passed on to the next generation. This necessity in telomeric division is provided by the activation of the telomerase enzyme [94]. In order to explain the relationship between telomere length and age, studies have been carried out in some cancer cells by cell culture of human fibroblasts in human cells of different age groups, and it has been found that telomere length decreases with increasing cell division rate and age [95].

Telomeres are extended by mechanisms that include telomerase-mediated reverse transcription, subtelomeric DNA amplification, and telomeric DNA homologous recombination. It eliminates the risk of uncontrolled telomere elongation such as end-to-end junction, premature aging and related disorders such as cancer. In addition, the mechanisms that determine different aspects of longevity, early replicative aging, and chronological aging appear to depend on the accuracy of DNA damage response and repair. For example, the investigation of effective and adequate regulation of telomere DNA damage response and repair by examining the molecular interactions between different telomere maintenance pathways in telomere care will shed light on the mechanisms of preventing telomere damage from environmental stresses during aging [96]. The answer to the question of whether we can have a young and alive body by increasing our telomere length has shown itself with the latest studies. It has shown that it can be reversed by increasing the telomerase ratio inside the cell and stopping aging. Researchers who cultured and then cloned telomerase genes in human cells; they reported that the cell continues to divide even after the aging point, with telomeres that stretch up to a thousand base pairs. It should also be known that a procedure performed only in this way carries a serious cancer risk [1,93]. Within all these data, it would not be wrong to say that these formations that form "happy ends" of chromosomes have the potential to sign a happy future for humanity [90].

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