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Review article

A Brief Overview of Telomeres and Telomerase in Aging and Cancer

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Abstract

Telomere is guanine-rich DNA sequence with a protein complex known as shelterin present at the chromosomal ends to protect it from destruction and is known to play a key role in aging. Telomerase is a telomeric DNA elongating enzyme, which comprises the central key components for telomeric DNA synthesis. The main objective of this review was to explore the structure and function of telomere and telomerase, and their intervention in aging, stem cells, and cancer cells. The induction of various telomeropathies and age-related diseases by Telomerase RNA component (TERC) impairment is well explained. Telomeropathies refer to bone marrow failures such as dyskeratosis congenita, aplastic anemia, etc. Telomere and telomerase have become the targets for age-related therapies, stem cell therapies, and cancer therapies. This article throws light on the telomerase-related therapies with natural compounds in regenerative medicine and cancer treatment. Telomerase inhibitors like nucleoside ligands and G_4 ligands are explained in the context of anti-cancer drugs. The association of TERT promoter mutations with cancer is discussed. We conclude that understanding telomerase mechanisms in stem cells and cancer cells paves a way to translate stem cells, cancer cells, and age-related research into effective therapies. The dynamic features of telomerase and telomeres make their structural and biochemical studies difficult. Hence, further meticulous studies can be done on single-molecule approaches to facilitate feasible and effective studies of telomerase and telomere component functioning.

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1. Introduction

Telomeres are complexes of repetitive DNA sequences that are rich in guanine and telomere-aging associated proteins at the chromosomal ends. The ends of the chromosomes are preserved and chromosome stability is endured by telomeres as they are required by all eukaryotic cells that are undergoing cell division. The non-coding DNA sequences of telomeres undergo destruction during the replication of DNA in each cell division. Cells undergo replicative senescence when the length of the telomeres is shortened critically. Telomere shortening is observed in somatic cells after adequate cell division [1]. Telomerase is an enzyme that is a complex of RNA subunit, a catalytic reverse transcriptase protein, and telomerase-associated proteins [2]. The enzyme telomerase is capable of replenishing non-coding DNA sequences which are lost during cell division. Telomerase is actively expressed by some cells like stem cells, germ cells, etc. The role of telomere and telomerase in protecting the chromosomes was discovered by Carol Greider, Jack Szostakand Elizabeth Blackburn [3]. They discovered that telomeres were repeated sequences and cap-like structures at the chromosome terminus, and the enzyme telomerase was a telomeric DNA elongating enzyme with its role in chromosomal protection. In 2009, they received the Nobel Prize in Physiology and Medicine for their discovery of telomeres and telomerase enzymes. In recent years, it has been evidenced that the telomere maintenance pathway by telomerase and its associated proteins has a role in various events. The activation of telomerase and changes in the expression of various factors influencing the telomerase expression have a major role in cancer development, aging, etc. [4]. It was found that the telomerase enzyme has two integral components called Telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), which are essential in therapeutic interventions. Mutations in these components lead to poor stem cell differentiation and carcinogenesis. The telomerase enzyme is one of the principal targets of cancer, stem cell, and age-related therapies. Besides the chemical drugs for the activation and inhibition of telomerase, even the active components from plant extracts can be used for the same therapeutic interventions. The purpose of this study is to better understand the telomerase mechanisms in stem cells and cancer cells, which paves a way to translate stem cell, cancer cell, and age-related research into effective therapies. The research is also focused on telomerase-associated therapies with natural compounds in stem cell, aging, and cancer therapies. The potentiality of telomerase inhibitors like nucleoside ligands and G_4 ligands to be used as anti-cancer drugs is also examined. The association of TERT promoter mutations with different types of cancer is focused and discussed in the following sections.

2. Telomere and Telomerase

2.1 Structure of telomeres

Telomeres encompass repeated G sequences, and they are bounded by manifolds of interacting protein complexes called the shelterin complexes [5]. The DNA of telomeres comprises TTAGGG double-stranded tandem repeats with G rich overhangs that are single-stranded, and the overhangs are present at the 3’ end, and are thus known as G tails. To avoid being recognized as a double-strand break (DSB), the G tail hides within the double-strand of telomere DNA and forms a displacement loop known as the D loop [1]. Due to enriched G content, the G rich overhang (single-stranded) of the telomere forms G-quadruplex. G-quadruplex structures play a vital role in the inhibition of telomerase-dependent telomere extension, telomere protection, and suppression of recombination. Telomerase and helicases are the end-processing enzymes that require an unwounded single- stranded G tail at the 3’ end of the telomere. The G-quadruplex unwinding by at

least 6 to 12 nucleotides is required, and this facilitates the extension of the telomere by telomerase, and the alternative lengthening of the telomere (ALT) mechanism [6]. In humans, the maintenance of telomeres relies on a massive network of protein complexes (shelterin) that are present in the telomeric region. This protein complex is also known as telosome. The shelterin complex or telosome is composed of 6 proteins, i.e. TRF1 & TRF2 (Telomeric repeat-binding protein), TIN2 (TRF1-interacting protein 2), RAP1, POT1 (Protection of telomeres 1), and ACD (formerly known as TPP1) [7]. The shelterin complex links the single-stranded and double- stranded telomeric DNA and the telomeric proteins are stabilized [1]. TRF1 was the first double-stranded DNA binding protein of the telomere discovered. The length of the telomere is negatively regulated by TRF1. TIN2 protein can be identified by TRF1 due to the Telomeric Repeat Factors Homology (TRFH) domain on it (absent in TRF2). TRF2 has a critical role in the negative regulation of telomere length as TRF1, and it also contributes to protection of the telomere end. These activities are carried out by TRF2 when its interaction occurs with various factors such as ERCC1/XRF, DNA repair MRN complex, Apollo, FEN1, and WRN [8]. Telomeres require TRF2 to form a t-loop (loop-like structure). TRF2 is also required to subdue ATM activation and non-homologous end-joining (NHEJ) of chromosome ends. RAP1 lacks the capacity to bind with telomeres directly. It depends on TRF2 for binding and localization on telomeres. It was predicted that RAP1 also participated in suppressing NHEJ, but the definite function of RAP1 remains unknown. TRF2 and RAP1 also bind to the internal sequences of telomeres and regulate transcription. RAP1 protein is conserved with Myb domain, BRCT domain, and RCT domain. TRF1 is insignificant for end protection of telomeres and it is also required for the synthesis of lagging strands during DNA replication [9]. POT1 has been proven to control telomerase-dependent telomere elongation and protect the ends of telomeres from ATR- dependent DNA damage response [10]. POT1 prevents ATR-dependent DNA damage by blocking the binding of replication protein A (RPA) to the single-stranded DNA of telomeres [11]. In the shelterin complex, the protein which acts as the central component is TIN2. It acts as a central component by having direct interaction with TPP1, TRF1, and TRF2. Any disruption in TIN2 leads to the significant reduction of telomere localization of all shelterin components and augmented ATR-mediated DNA damage responses [12]. Among the proteins of the telosome complex, TIN2 and ACD play important roles in the recruitment of telomerase to the telomeres [1].

2.2 Telomere function

Telomere defends every eukaryotic chromosome terminal region against chromosomal recombination, chromosomal fusion as well as degradation of DNA in the terminal sites. Telomeric DNA length in autosomal cells in eukaryotic organisms ranges from 5 to 20 kb based on the type of organ and the proliferative and replicative status of each and every cell and cell age. During the mechanisms of cell division and DNA synthesis, the length of telomeres gets reduced because of incompleteness in linear chromosome replication. This biological occurrence is termed as an end-replication problem. To avoid destruction by exonucleases, the telomere 3′ single-strand overhang folds back into the D-loop of duplex telomeric DNA to form a protective T-loop. Here the T-loop is further reinforced with TRF2 and the other telomeric DNA-binding proteins that are part of shelterin. Mammalian telomeres possess lengthy tandem arrays of double-stranded telomeric TTAGGG repeats wrapped by the telomeric DNA-binding proteins such as the TRF1 and TRF2 proteins.

Maintaining telomeres necessitates an important enzyme called telomerase as well as a group of telomere-associated proteins [13]. The DNA damage response (DDR) is a cumulative network of DNA repair, DNA-damage tolerance pathways, and cell cycle checkpoints. Any epigenetic and genetic anomalies and also other biochemical abnormalities in telomere-associated

proteins, epigenetic enzymes, and telomerase can lead to abnormal telomere length, telomere end-end fusion, telomere damage-induced foci (TIF), and structural changes that ultimately result in cell cycle arrest, genome instability, and cell senescence. In humans, telomere dysfunction is a biochemical mechanism observed in cancer development, bone marrow failure syndromes, and leukemia [14].

2.3 Telomere position effect (TPE)

Telomeres protect the chromosomal ends by capping them, and these telomere sequences are reduced in each cell division because of end-replication problems. The expression of genes that are located close to the telomeric region is affected by the telomere and this phenomenon is termed as the telomere position effect. TPE sometimes results in the silencing the genes near the telomere locus, especially in yeast. TPE was first identified in *Saccharomyces cerevisiae* where it was observed that RNA polymerase II gene expression incorporated adjacent to the telomere region was suppressed. Other genes are also found to be affected by these telomere regions. ISG15 is located on the human chromosome 1p36.3 1 Mb far from the telomere locus and has a higher expression in aging cells or older-aged cells [15]. Scientists proved that HeLa cell lines that have a luciferase reporter located adjoining to a telomere that is newly formed express 10 times lower luciferase than that of control cell lines, which are produced by random integration. It was found that TPE not only affects genes near or adjacent to telomeres but also affects genes situated far from telomeres, even up to 10 Mb, by means of long-range loop formation. This type of TPE is called a telomere position effect over long distances (TPE-OLD).

2.4 Role of telomere in aging

The reduction in telomere length is the major underlying cause of aging. Short telomeres trigger cell viability loss, and cell as well as chromosome senescence [16]. Critical telomere repeats are very necessary to ensure good telomere function, and to prevent the activation of DNA damage pathways that lead to cell death (or replicative senescence). Dyskeratosis congenita is a kind of progressive and congenital telomeropathy. Dyskeratosis congenita is due to a genetic malformation in which mutations in the telomerase RNA component, i.e. TERC impair the maintenance of telomere length and hence result in cellular senescence elevation that paves the way for the clinical manifestations of premature aging symptoms. Recent findings suggest that the ability of hematopoietic stem cells to proliferate lessens with age as there is a gradual DNA loss in the telomeric region, i.e. from the human chromosomes terminal sites with each cell division and thus results in cellular senescence. All the human somatic cells compiling stem cells also demonstrate gradual telomere length shortening with each and every cell division. This occurs until the telomeres reach a significantly reduced length and stimulate a signal for DNA damage which is often termed as cell aging or replicative senescence [17]. Thus, it can be said that telomeres act as chromosomal caps to preserve the ends of the chromosome from DNA damage and also act as a measurement of the mitotic age of a eukaryotic cell. The time point at which a particular chromosome terminal site becomes uncapped is dependent on the telomere shortening rate in any tissue or cell. Telomere that is the shortest is highly likely to take less time to uncap the chromosome, and this is significant in chromosomal stability as well as cell viability, and can signal senescence onset.

2.5 Structure of telomerase

Telomerase comprises the following central key components: RNA subunit of telomerase (hTR or TERC), a catalytic reverse transcriptase protein, or a catalytic subunit of telomerase (hTERT), and

telomerase-associated proteins [2]. The catalytic reverse transcriptase protein has been categorized into three important structural and functional domains. They are, TRBD (a telomerase RNA-binding domain), a reverse transcriptase domain, and TEN (a telomerase essential amino terminal domain) in humans. Telomerase has been observed to involve a dimeric complex, which has two active sites, with two hTERTs and two hTRs [17, 18]. The sequence and length of the RNA subunit of telomerase vary among different organisms. The RNA recognition proteins which are accommodated by the RNA subunits of telomerase are distinct. Some of the elements such as the CR7 domain, the box H/ACA (CR6/CR8) domain, the CR4/ CR5 domain, and the CR2/CR3 domain (pseudoknot domain) exist in the telomerase RNA with significant functions [19]. The hTR serves as a template for the telomeric DNA to synthesize poly G tails. This template is present at the 5' end. The transcription of hTR is carried out by RNA polymerase II to yield a mature telomerase RNA with 451 nucleotides, and this occurs at the 3' end. But, the hTR H/ACA domain is absent in lower eukaryotic organisms. The box H/ACA domain of telomerase RNA has been observed to be indispensable for telomerase activity, especially the processing of hTR at the 3' end, and its accumulation. Domains that are present in hTR molecules serve as recognition sites for various hTR binding proteins. The hTR binding proteins are as follows: hTERT, La, hnRNP C1/C1, L22, hStau, hNHP2, hNOP10, hGAR, and dyskerin. These RNA binding proteins play a vital role in upholding hTR accumulation, mutation, and stability. The catalytic subunit of telomerase (TERT), i.e. the reverse transcriptase enzyme of telomerase has reverse transcriptase motifs which are located in the C-terminal of the proteins. It has T-motif, which is the preserved telomerase-specific region, and it is located at the N-terminal position of the reverse transcriptase motifs. This reverse transcriptase enzyme of telomerase has highly conserved essential domains in the large N-terminal region [20]. Telomerase requires telomerase-associated protein for efficient action of telomeric DNA. P23 and HSP90 (heat shock protein 90) are the additional proteins that are helpful in assembling TERT with hTR. These telomerases associated proteins are thought to serve as binding sites for other proteins. Hence, this arrangement promotes the transportation of TERT molecules to the nucleus [21]. The combination of various enzymes, domains, motifs, and proteins aid the telomerase for well-organized binding with telomeres and elongation of its repeated sequences of DNA at the 3' end. Figure 1 presents the schematic outline of the structure of telomerase and the extension of telomeric DNA by telomerase.

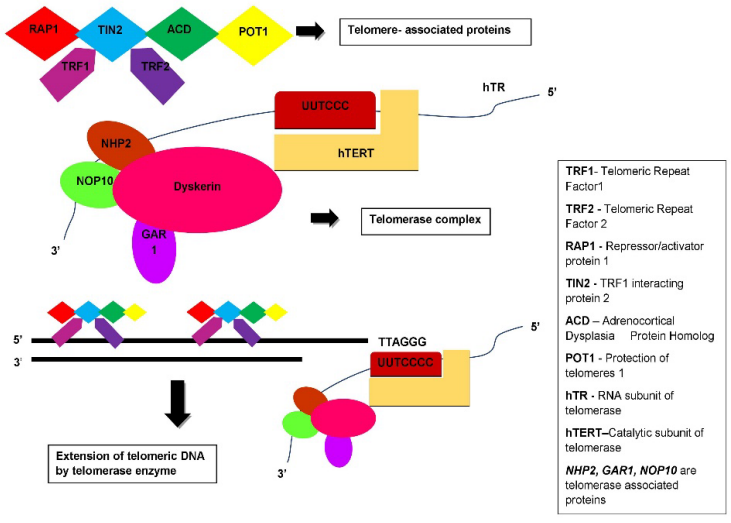

Figure 1. The six protein subunit complex of the telomere called shelterin, aids in the extension of telomeric DNA by the telomerase complex

2.6 Natural telomerase

In humans, the majority of normal somatic cells do possess telomerase. The abnormal activation of telomerase triggers tumor genesis and implicated aging, and the major cause is depletion of the telomere. The presence of this enzyme in 90% of cancerous cells led the pharmaceutical industry to design drugs that specifically targeted the telomerase enzyme. Nowadays, commercially produced synthetic products are used in chemotherapy of cancer in spite of the occurrence of side-effects or complications in patients with cancer. Therefore, it is essential to explore the beneficial effects of natural products obtained from various medicinal plants on various cancer cells, and to research the potential anti-cancer therapeutic effects of these natural products. In fact, natural products can be taken normally in the human diet as they are safe to consume, edible, and have higher acceptability among individuals [22]. The activity of telomerase enzymes in cancer cells is usually inhibited by numerous natural products. This inhibition is associated with cell viability reduction [23].

3. Role of Telomerase in Human Cells

Telomerase activity can be measured by telomeric repeat amplification assay in the cell lysate (*in vitro*). This telomeric repeat amplification assay was developed particularly to measure the level of expression of telomerase enzymes in various types of cells [24]. Most somatic cells which undergo differentiation show a lack of telomerase activity, and this was confirmed using the above-mentioned assay. The expression of telomerase is relatively high in male and female reproductive organs called the testes and ovaries. This is likely to facilitate the inheritance of reliably longer telomeres in the next generation. The expression of telomerase is undetectable in neonatal stem cells. Its expression begins to decline in the later blastocyst stage but telomerase remains functional at the early embryonic developmental stage [25]. In stem cells, the activity of telomerase is weak, but it extends the proliferative capacity of stem cells [26]. The expression of the telomerase enzymes describes the proliferative ability of cells and the homeostasis of the length of the telomere. Telomere elongation at the 3' end of its DNA sequence occurs in the presence of telomerase enzymes, and it is not a single-step process; it happens in multiple steps. Telomerase functions actively in the presence of telomerase-associated proteins. This process is exactly and critically regulated. The steps include the coordinated synthesis of the C-strand, substrate recognition, ribonucleoprotein assembly, post-translational modification of hTERT, nuclear transport, processing, and maturation. Telomere length homeostasis can be maintained when the active telomerase enzyme has been recruited efficiently to every telomere when its length gets reduced [27]. In the TERT heterozygous cells of the mouse, it has been demonstrated that shorter telomeres have a short stretch of telomeric DNA binding proteins, and this negative regulation paves a way for telomerase recruitment. The same mechanism takes place in yeast.

3.1 Regulation of telomerase in human cells

In human beings and other mammalian cells, telomerase recruitment occurs in the S phase of cell division [28]. The POT1-ACD complex of the shelterin is known to get associated with telomerase soon after its recruitment with telomeres. This POT1-ACD complex plays a major role in enhancing telomerase activity and processivity, facilitating translocation of a template, and reducing the dissociation rate of the RNA primer. TIN2, one of the proteins of the shelterin complex, aids in the recruitment of telomerase [29]. In the cell cycle during the S phase, phosphorylation of ACD or TTP1 is aided by Cdk1 and kinase. It is believed that phosphorylation of TPP1 facilitates extensive stability of its interaction with hTERT but this phosphorylation-mediated interaction mechanism is

debatable. There is the possibility that TIN2 may raise the telomerase recruitment. TRF1 and TRF2 are the proteins of the shelterin complex which negatively regulate the extension of telomeres by telomerase. TZAP is an alternative DNA binding protein of the telomere, which is equally exclusive to the binding of TRF proteins. The TZAP protein excises the t-loop of the telomere to trim it. Telomerase processivity and retention are carried out by the TEN domains of telomerase. The TIN2 protein of telomeres gets associated with the heterochromatin proteins that regulate the cohesion and condensation at telomeres [29]. The 3' overhang of single-stranded telomeric DNA at its distal end, designs the substrate for the telomerase, once it reaches the telomere. Telomerase RNA adds one repeat of DNA at the single-stranded 3' distal end of telomeres. In this process, the telomerase RNA sequence acts as a template that is complementary to the telomeric DNA. Telomeric DNA extension takes place once the template region which is present on the telomerase RNA anneals with the substrate formed at the telomeric DNA and leads to the formation of DNA/RNA hybrid. Then the telomerase enzyme moves its RNA template further on the substrate to add one more telomeric repeat at the 3' distal end of the telomere. This process is termed RAP (repeat addition processivity), which refers to the capability of the telomerase enzyme along with its associated proteins to add repeats of DNA sequences simultaneously until it reaches the endpoint without dissociating from the substrate formed on the telomeric DNA [30]. The appropriate mechanism of telomere repositioning needs to be explicated in the future. Multiple factors are involved in telomerase processing, telomerase activation, telomerase recruitment to the telomere, synthesis of 3' overhang, and repositioning of telomerase RNA. Once the telomere is elongated, it has to terminate the telomerase action on it. This is carried out by the CST complex. The CST complex (TEN1, STN1, CTC1) of telomeres is known to inhibit the telomerase processivity and terminate telomere elongation [31]. There are various other factors that inhibit telomerase processivity as follows: (i) Lack of link or interaction amidst telomere lagging strand synthesis and telomere extension; (ii) the G-quadruplex formation inhibits the action of telomerase on telomeric DNA. The POT1-ACD complex has a key role in averting the formation of G-quadruplex, when the telomere is needed to be elongated by the action of telomerase [32]. Further studies need to be done, to understand the detailed mechanisms of telomerase binding, telomeric DNA elongation, and termination of telomerase activity and processivity, and to know the exact factors which are involved in these processes.

3.2 Role of telomere and telomerase in stem cells

The telomerase activity in somatic cells except for lymphocytes and stem cells is minimized after birth because of the phenomenon in which the length of the telomere gets reduced with every cell division. Taking the reality that stem cells have high proliferative ability and self-renewal potentiality into consideration, it was noted that there must be a special mechanism that regulates telomere length through stem cell divisions. Indeed, minimum telomerase activity was reported in adult stem cells including hematopoietic as well as non-hematopoietic stem cells like skin, neuronal, intestinal crypt, mammary epithelial, pancreas, mesenchymal stem cells (MSCs), adrenal cortex and kidney stem cells. An important fact that was observed was that telomerase and telomere activity in mouse and human cells vary and the telomerase and telomere status in stem cell populations varies between mice and humans. In adult stem cells, the telomerase activity level is undetectable or minimum and it is elevated in progenitor cells that possess an enhanced reproducible activity but cannot stably maintain their telomere length.

Many scientists and researchers have experimented on the levels of telomeres and telomerase in different types of stem cells of human beings [33]. It was reported that embryonic stem cells and cancer stem cells have high telomerase levels leading to well-maintained telomere length. In contrast, other stem cells like neuronal stem cells, liver stem cells, hair follicle stem cells

have lower levels of telomerase, and thus the telomere length is not constant, and the reduced length of the telomeres for every cell division is comparatively greater than embryonic stem cells and cancer stem cells.

Because of the end replication problem phenomenon, the telomere shortens its length during stem cell aging and also during differentiation. However, this effect can be reduced through telomerase enzyme action and the ALT mechanism, and thus telomeres are lengthened to sustain self-renewal capacity [34]. Enhanced proliferative tissues, and particular cells subjected to demanding proliferative stimuli have ahigh demand for telomerase activity in order to carry out their physiological functions and biochemical mechanisms without compromising cell viability through exhaustion of telomeres. The capacity of hematopoietic stem cells to maintain the activity of telomerase enzymes after differentiation and proliferation stimuli may be vital in the cell-renewal actions that regulate blood cell turnover throughout the lifespan of a person. Telomerase is a cellular reverse transcriptase and RNA-containing enzyme which adds DNA to the chromosomal ends. Telomerase aids in regulating and controlling the genome integrity in proliferating progenitor cells as well as in embryonic stem cells which are derived from quiescent stem cells. Telomerase is silent in almost all human tissues and it is expressed in a minimum number of cell types like replicating spermatocytes (male germ-line cells) and a subset of progenitor cells. There is strong proof that progenitor daughter cells express elevated telomerase levels while the other daughter stem cells enter a quiescent state and do not express the telomerase enzyme. The reason and the physiological activity behind the suppression of telomerase activity in quiescent stem cells are yet to be found.

3.3 Telomeropathies in stem cells

Telomeropathies are a kind of rare disorder. Telomerase complex genetic mutations often result in impaired control of telomere length mechanisms, and thus this impairment results in telomeropathies. Telomeropathies that appeared in *Homo sapiens* patients commonly present with a great range of clinical symptoms [35]. The most widespread and severe is bone marrow failure [36]. In such cases, the patients undergo HSC transplantation therapy [37]; however, the long-term survival rate is only 28% [38]. Telomeropathies depicting clinical manifestations with bone marrow failure syndromes, for example, dyskeratosis congenita, myelodysplastic syndromes, aplastic anemia, etc., deprive effective and specific treatments. Here the telomeropathies are clinically managed with adjuvant therapies like hormonal, cytokine, or antioxidant and immuno-suppressive therapies.

The component of the telomerase complex, TERC expression when decreased, resulted in weakened and damaged hematopoietic stem-cell (HSC) differentiation. Deregulated telomere attrition and *TERC* deficiency ultimately lead to loss of hematopoietic stem-cell renewal capacity and also lethal bone marrow failure. TERC impairment elevates the generation of reactive oxygen species and the expression of cellular senescence markers. This was proved by an experiment with a telomerase function imbalance model by means of shRNA [39]. shRNA knockdowns the expression of TERC in induced pluripotent stem cells (iPSCs). shRNA is introduced into the host cells through viral vectors which are an effective way to facilitate stable integration of shRNA, and this introduction causes long-term knockdown of the gene under study. TERC impairment elevated the generation of reactive oxygen species (ROS) and cellular senescence marker expression and led to weakened hematopoietic stem-cell differentiation. Interestingly, the length of the telomere sequence was not affected in shTERC knockdown induced pluripotent stem cells (iPSCs), leading to a discovery that this malfunctioning differentiation phenotype is maintained by non-telomeric functions of telomerase which showed that these phenotypes are directly linked to telomerase activity but independent of telomere length [39].

Savage and Niewisch [40] found that dyskeratosis congenita, which is a dominant autosomal genetic disorder, is associated with the genetic mutations underlying TERT or TERC, which are integral components of the telomere complex. The mutations in this disorder are thoroughly analyzed and understood, as they conventionally affect the expression of *TERC* or TERT, which are integral parts of the telomere complex. Mutations in telomerase integral components like TERC and TERT were reported in patients suffering from dyskeratosis congenita as well as aplastic anemia. Both ailments were identified and analyzed as bone marrow failure and a few skin deformities due to poor differentiation capacity of the hematopoietic stem cell compartment. Figure 2 presents a schematic outline of the induction of telomeropathies and age-related diseases.

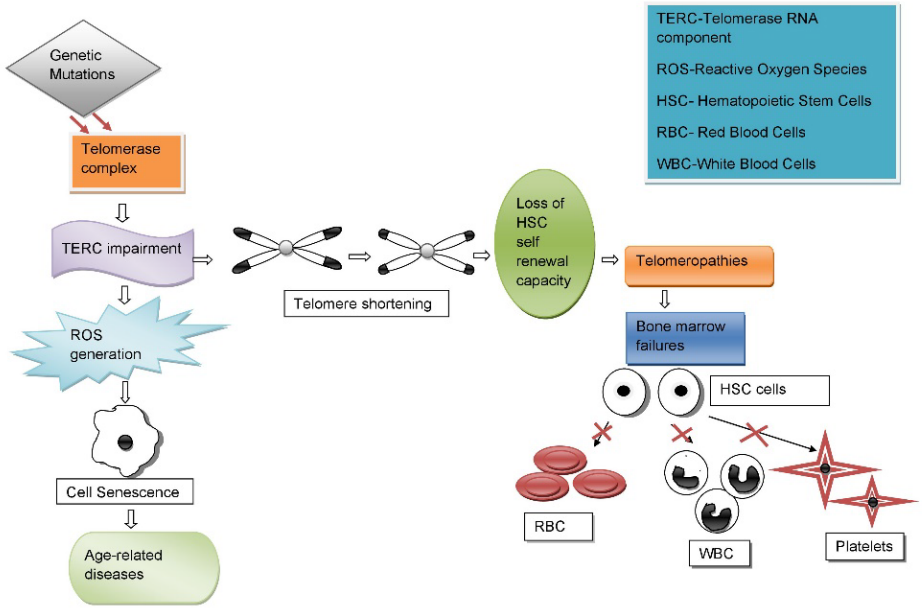

Figure 2. Genetic mutations in the telomerase complex cause TERC impairment which leads to ROS production and reduction in telomere length to cause age-related diseases and telomeropathies.

The state of telomeres has an effect on stem cell pluripotency which was proved that "telomeric chromatin" is highly effective and active in pluripotent stem cells (PSCs) when compared to other autosomal cells, and turns repressive during the cell differentiation process [41]. On the other hand, reprogramming of these cells into PSCs can alter the telomeric chromatin from repressive state to open state which is similar to that of a stem cells. However, the mechanism behind this alteration should be explored. It can be concluded that the molecular dynamics of telomeric chromatin play a vital part in regulating the pluripotency of human stem cells. Controlled regulation of telomere length along with telomere-binding proteins is vital for genomic stability and telomere function in pluripotent stem cells and human somatic stem cells.

In an analysis during reprogramming of blastocyst cells to embryonic stem cells (ESCs), telomeres were also elongated along with other modifications [42]. It was hypothesized that cells with lengthy telomeres have greater pluripotency capacity and are positively selected from a heterogeneous population. Single-cell analysis technology has been identified and greatly applied

to examine the heterogeneity of cells. A technique that simultaneously measures telomere length as well as studies the transcriptome in the same cell is currently available. This technique is made possible by a method called scT&R-seq analysis. The scT&R-seq analysis gives the data regarding telomere length along with RNA-sequencing analysis [43].

The scT&R-seq analysis revealed that human embryonic stem cells (ESCs) with short telomeres showed more features associated with differentiated cell subsets, and conversely, ESCs with greater telomere length possessed pluripotency. Telomere length is important in stem cell differentiation. Induced pluripotent stem cells with long telomeres were found to have mostly differentiated into endodermal and mesodermal lineages, much like progenitor cells of the heart [44].

3.4 Role of telomere and telomerase in cancer stem cells (CSCs)

A deep and clear knowledge of telomerase as well as telomere dynamics in normal stem cells and cancer stem cells can give a clear picture of the differences between normal and cancer stem cells. There is evidence that most leukemia stem cells are not quiescent and also possess the characteristics of self-renewing committed precursors or progenitors, in contrast to quiescent normal human stem cells [45].

Cancer stem cells must bypass senescence and the growth arrest state in the cell cycle, and they have short telomeres but upregulated levels of telomerase. For example, in most cases of preneoplasia and prostatic intraepithelial neoplasia (PIN), the key cells present were found to have very short telomeres. This finding led to the hypothesis that tumor-initiating cells derive from transitory amplifying cells that have undergone persistent inflammation. Gradual telomere shortening leads to chromosome end fusions, chromosome end associations, breakages during every cell cycle, and anaphase bridges. This results in genomic instability, which is a feature of the majority of cancer cell types. It also suggests that forceful inhibition of telomerase can be an efficient anti-cancer treatment that can target massive cancer stem cells as well as cancer cells [46]. There is the explanation that budding cancer cells originate from highly self-renewing telomerase-competent cells and mutations in the promoter of TERT to control telomere length are not required. Hence, cancers evolving from such highly proliferating and replicating cells tend to have low levels of mutations in human TERT promoters. However, cancer-initiating cells or cancer stem cells with less self-replicating potentiality need to overcome the short-telomere-dependent proliferative barrier via TERT promoter mutations.

The role of telomerase in cancer stem cells development throws light on development and designing new strategies for cancer therapies targeting cancer cells and also cancer stem cells. This also signifies the importance of stem cell biology integration with cancer biology and other cancer studies.

3.5 Role of telomeres and telomerase in cancer cells

The repetitive protein-DNA complexes at chromosomal terminal sites, which are termed as telomere regions, are very critical in the progression and survival of cancer cells. Cancer cell survival depends on telomeres, and telomeres are maintained by telomerase enzymes. The biochemical mechanisms underlining telomere length regulation and telomerase expression include transcriptional, epigenetic regulation, and post-transcriptional regulation, and an extensive understanding of these biochemical reactions may give rise to significant biomarkers which can help in the early detection of cancer and disease, prognosis determination, and design of treatments. Cancer cells reach a state of high proliferation rate by upregulating or activating the normal human TERT (hTERT) gene that codes for the enzyme telomerase. Human TERT (*hTERT*) gene is generally silenced in most somatic cells, but in contrast, it is notably expressed in approximately 90% of tumors in human beings. The

knowledge of the mechanisms of *hTERT* activation is under elucidation. Some of the underlying mechanisms of *hTERT* activation include the alternative splicing of *hTERT* pre-mRNA, *human TERT* promoter mutation, *hTERT* amplification, telomere position effect (TPE) disruption, and epigenetic changes. The mutations in promoters that are located in either −124 base pairs or −146 base pairs upstream from the translation start site of TERT are reported to be the reason behind an unconventional rise in telomerase expression. Moreover, other molecular mechanisms that control and maintain the expression of human *TERT*, as well as telomerase assembly, are currently under intense investigation and study. Studies and therapies using strategies targeting telomerase inhibition has the potentiality in paving a way in telomere length shortening and cancer cell mortality. Many telomerase inhibition approaches involve the use of antisense oligonucleotides, small-molecule inhibitors, G-quadruplex stabilizers, immunotherapy, etc. In addition, numerous anti-telomerase treatments are being analyzed in clinical trials of different cancer types. Recent developments in the fields of telomere and telomerase studies implicate cancer research and cancer therapies.

Non-coding region mutations that occur in the *hTERT* promoter are one of the most commonly occurring mutations, and the frequency and level of such mutations differ among various types of cancer. Cancers like melanoma, myxoid liposarcoma, dermal sarcoma, urothelial cell carcinoma, glioma, liver cancer, and skin carcinomas have high numbers of *TERT* promoter mutations [47], and low levels of *TERT* promoter mutations are seen in gastric cancer, gastrointestinal stromal tumors, non-small-cell lung cancer, and pancreatic cancer [48]. *TERT* promoter mutations were not observed in prostate cancer portraying that malfunctions inside the *TERT* gene promoter do not play a key role in cancer formation. *TERT* promoter mutations have been found in almost all stages of the solid tumors. This finding leads to the observation that *human TERT* mutations are usually an initial episode in cancer formation [49]. Other mutations were also reported. It was observed that the protein ARID1A suppresses *TERT* expression, and thus mutations in ARID1A causes this protein not to function efficiently, and increasing *TERT* expression [50, 51]. The PIK3CA/AKT/NF-kB pathway enhances TERT activity at the protein level via TERT phosphorylation by AKT in ovarian and breast cancers [52]. *TERT* expression is maintained by both transcription factor status and epigenetic factors. DNA methylation in the *TERT* promoter region has been studied for two decades and hypermethylation in the TERT promoter leads to high expression. Hence it was noted that TERT expression can be modulated based on the methylation of DNA. Based on epigenetic conditions, DNA can be hypermethylated or hypomethylated. Apart from TERT promoter mutations, some associations with some exogenous regulators including tumor viruses encode proteins that stimulate TERT transcription. Some of the key viral proteins that affect TERT transcription are Epstein-Barr virus, human papillomavirus cytomegalovirus and hepatitis B virus, which are responsible for targeted activation of TERT transcription acting in virus-mediated carcinogenesis.

3.6 Anti-telomerase therapeutics

The main objective of anti-telomerase therapeutics is to selectively induce cell death along with apoptosis in cancer cells with less effect on other normal cells in humans [53]. Enormous methods were discovered and developed to achieve this objective include the development of vaccines, antisense oligonucleotides, and small-molecule inhibitors to target hTR or hTERT. The GRN163L (oligonucleotide imetelstat) was reported to be the most propitious telomerase inhibitor. Bryan *et al*. [54] found that BIBR1532 which is a telomerase inhibitor, binds to the TERT in the thumb domain thus disturbing RNA-TERT interaction to downregulate the transcription. Additionally, G-quadruplex stabilizers and tankyrase inhibitors, and HSP90 inhibitors, target telomere and telomerase assembly. T-oligo has been investigated and it has the ability to selectively destroy

tumor cells. Tankyrase inhibitors have significant involvement in the homeostasis of the telomere, WNT/β-catenin signaling, and mitotic spindle formation. HSP90 inhibitors have an important role in protein degradation, signal transduction, and intracellular transport. T-oligo is the DNA oligonucleotide that is homologous to the 3′ overhang location of telomeres and it induces cytotoxic effects with DDR [55].

Telomerase is an attractive target for designing telomerase-based immunotherapies. In cancer and tumor cells, proteasomes degrade the telomerase, leading to the creation of protein peptides or fragments of telomerase that are expressed as antigens on the surface of cancer cells by the human leukocyte antigen (HLA) class I pathway. Here, such telomerase antigenic epitopes can be targeted by cytotoxic T cells to destroy the cancer cells. Some of the vaccines targeting hTERT such as GV1001, GRNVAC1, and Vx001 were considered to produce anti-telomerase responses related to the immune system in tumor or cancer-affected patients [56]. GV1001 is a 16-amino-acid. It is a human TERT peptide with amino acid sequence EAR-PALLTSRLRFIPK and Vx001 vaccine is a cryptic peptide -based vaccine in which functional peptides are concealed in protein structures containing human TERT with an amino acid sequence YIFY RKSV. The results of human trials have elucidated that these vaccines target the enzyme telomerase, induce responses in the T cell immune system in telomerase-positive tumors, have less impact on other normal human cells, and cause no problem with autoimmune diseases [57]. Another important strategy is to integrate an altered nucleotide into the telomeres, resulting in a malfunction of the telomere region and swift and efficient cancer cell mortality [58].

3.7 Activation of telomerase by natural compound

Progressive telomere shortening can be achieved by a proliferation of telomerase negative cells. Though cellular senescence mechanisms protect against cancer, subsequent dysfunction can lead to tumorigenesis. The length of telomeres expands due to the binding of the enzyme Telomerase. Geron Corp developed a single molecule-telomerase activator, TAT2 (cycloastragenol), and TA therapeutics [59]. Cycloastragenol has anti-aging properties and it is a powerful telomerase activator in neurons while screening *Astragalus membranaceus*. Studies showed that cycloastragenol elongates short telomeres and increases the health span of adult mice [60]. However, there was no carcinogenesis observed due to its elongation. It also got licensed as a nutritional supplement that is commercially available as TA 65 (TA sciences, Geron Corp.) [61].

Some phytochemicals like resveratrol and genistein tend to activate telomerase enzymes - telomerase. Genistein, found in soybean, is a natural isoflavone. In breast MCF10AT benign cells and MCF7 cancer cells, Genistein restrains the transcription of hTERT, and it also decreases the activity of telomerase in prostate cancer cells [62]. Ouchi *et al*. [63]. demonstrated that the genistein downregulates the expression of hTERT and c myc mRNA in prostate cancer cells but it was found that physiologically obtainable concentrations of genistein can improve the activity of telomerase in prostate cancer cells; however, low concentrations of Genistein may activate and inhibit telomerase activity at higher treatment concentrations. The effects of *Ginkgo biloba* on telomerase activity are still under investigation. Dong *et al*. [64] suggested that *Ginkgo biloba* extract can increase telomerase activity in endothelial progenitor cells. Bodnar *et al*. [65] analyzed two telomerase-negative normal human cell types. The vector-transfected cells encode to the human telomerase catalytic subunit. Telomerase expressing clones had elongated telomeres and showed reduced senescence signs.

Replicative senescence tends to lessen various physiological functions in tissues, and thus paving the way for the induction of chronic diseases [66]. The restoration of telomerase immortalization in somatic tissues is a significant alternative to address chronic diseases [67]. Some of them are histone deacetylase inhibitors (hDAC), and others are estrogen receptor agonists, the

latter acting by Akt-mediated phosphorylation. Plentiful medications with primary targets other than telomerase additionally have an effect on hTERT at the transcriptional or post-translational level and included are signaling pathways that elevate hTERT expression, and also the activities of MAPK/ERK1/2, PI3/Akt, and the Wnt/βcatenin pathway. In lymphocytes, clonal expansion naturally activates telomerase activity by means of enzyme phosphorylation and consequent nuclear translocation. This function reduces with age and prompts exhaustion of memory cells and can be re-established by direct interaction with the telomerase activating signaling pathways or the telomerase holoenzyme.

Modulation of the intracellular location, or the sequestration of telomerase, is another level of regulation of telomerase activity, implicating telomerase localization as a possible focus for pharmacotherapy [47]. Between the cytosol and nucleus, telomerase is translocated. In mitochondria, hTERT is present with insignificant physiological importance. The pharmaceutical significance of telomerase reconstruction was chiefly discussed in terms of therapies for health ailments with the damaged enzymatic activity of telomerase such as aplastic anemia, dyskeratosis congenita, and so on. Good additional benefits included were the production of epithelia for wounds or burns, chondrocytes for arthritis treatment, endothelial cells for blood vessels, osteocytes for bone defects, hematopoietic cells for bone marrow transplants, and the replacement of immune cells [68]. Using this technique, human blood vessels have been devised *in vitro* already. Transitory telomerase activation has the potential to address other chronic diseases, for example, atherosclerosis, cardiac muscle ailments, bone marrow failure, and immunodeficiency diseases, liver disease, pulmonary fibrosis, cataract, degenerative cartilage ailments [69], rheumatoid arthritis, organ transplantation, therapies linked with the increased senescent cell formation like HIV, cancer therapy, etc. Cartilage tissue engineering mainly targets cartilage defects. Bovine TERT modified bovine adrenocortical cells was verified [70] and these modified cells can be transplanted into severe combined immunodeficient (SCID) mice. The cell clones act just like their normal counterparts and lead to the formation of functional tissue after transplantation. This tissue is histologically similar to tissue formed from normal cells and shows a similar rate of cell division, implying a therapeutic role of telomerase in xenotransplantation. The development of telomerase activator induces hTERT and/or hTR expression, enhances enzyme activity, or influences cellular location with the association between telomere length and aging. The aim of this particular way is to reverse normal cellular aging as well as to treat aging symptoms.

Inhibition of telomerase in cancer treatment in combination with traditional chemotherapeutics is an imperative choice but activation of telomerase can address age-related ailments and can assist AIDS patients (i.e., patients affected with HIV), where lymphocytes have been blocked from dividing or proliferating. However, the long-term effects of regulating telomerase activity either positively or negatively are still to be verified. It was observed that telomerase activity inhibition may lead to unfavorable side effects in normal stem cell function and immune response as stem cells and immune cells have been enhanced by the activity of telomerase enzymes to facilitate frequent cell division. A deeper understanding of the regulation of telomerase enzymes in normal cells is necessary for the development of both telomerase activators as well as inhibitors [71].

3.8 Inhibition of telomerase

Destabilization as well as deactivation of telomerase activity by natural product has become a challenge in the targeting of cancer cells. Nowadays, the biggest downside of chemotherapy drugs is that they are not specific, and affect cancer cells as well as normal cells. Differentiation between normal and cancer cells can be enabled by telomerase inhibition as detection of telomerase is not found in the normal cells [52]. Targeting telomerase in malignant growth is considered to be safe

due to the differences in telomere length, telomeric expression, and cell kinetics of normal cells and cancer cells.

Inhibition of telomerase paves the way to potential targeted therapy for cancer. There is a high focus on the development and standardization of potential telomerase inhibitors worldwide. There are various types of telomerase inhibitors like nucleoside analogs, G-4 stabilizers, and these are explained below.

3.8.1 Nucleoside analogs

Hernandez-Sanchez *et al*. [72] performed a direct *in vitro* telomerase activity assay in the presence of deoxynucleoside triphosphates (dNTPs) along with each indolyl-2′-deoxynucleoside triphosphate analog and concluded that there was a significant but slight reduction in telomerase expression when *Tribolium castaneum* telomerase reverse transcriptase was administered with 6-nitroindolyl-2′-deoxyriboside 5′-triphosphate analog. 5-methylcarboxyl-indolyl-2′-deoxyriboside 5′-triphosphate (5-MeCITP) was tested on the *Tribolium castaneum* telomerase reverse transcriptase (TERT) model. The study of the crystal structure of 5-MeCITP bound to the *Tribolium castaneum* TERT revealed that the carboxyl group of 5-MeCITP helps to position the methyl moiety at a distance away from the ribose ring to make the methyl fit into a specific hydrophobic pocket of TERT. It was observed that 5-MeCITP produced a significant decrease (more than 80%) in telomerase activity [73]. This study also included the testing of other telomerase inhibitors such as deoxyadenosine triphosphate, deoxyguanosine triphosphate, 4-nitroindolyl-2′-deoxynucleoside, 5-methylindolyl-2′-deoxyriboside 5′-triphosphate, 5-aminoindolyl-2′-deoxyriboside 5′-triphosphate, 5-ethylene indolyl-2′-deoxyriboside 5′-triphosphate and 5-carboxylindolyl-2′-deoxyriboside 5′-triphosphate of which 5-MeCITP was found to have a high potency to reduce telomerase activity over the mentioned nucleotide analogs.

The telomerase inhibition property of the nucleotide analog, Zidovudine, was applied to target and treat retroviral infections. Zidovudine leads to cell cycle perturbations and telomere length reduction [74]. The experiment conducted by Francke *et al.* [75] showed that the administration of Zidovudine was associated with increased cytotoxicity even in non-cancerous cells, which acts as a barrier to its clinical applications. However, the efficiency of 5-MeCITP and Zidovudine were similar in their inhibiting telomerase activity.

3.8.2 G_4 ligands

G-quadruplex structures or G4 structures consist of four consecutive guanines linked with Hoogsteen hydrogen bonds [76]. The structural stability is maintained by each guanine which acts as a donor and acceptor for two hydrogen bonds. G-quadruplex structures are located in DNA as well as RNA transcription factor binding sites. Promoters and telomeres are rich in G-quadruplex structures.

The G4 structures at telomeres have the ability to change telomerase binding affinity. Thus, any alteration can block telomerase activity [77]. Hence G4 structures are potential targets in telomere targeted drug development. Ligands specific to G4 structures are potential telomerase inhibitors. For example, Telomestatin leads to telomerase inhibition. Derivatives of diamidoanthraquinone also show similar properties. RHPS4, a G4 ligand, destroys the shelterin complex (telomere protectant) causing impaired function of the telomere. However, the telomere is repaired in the PARP1 dependent pathway. Therefore, administration of cancer cell lines with RHPS4 and PARP1 inhibitor (GPI-15427) in combination produced better results, i.e. greater reduction in cancer cell growth [78].

Among the less common sources of telomerase inhibitors were found in marine-based organisms. In 2003, the first marine-derived telomerase inhibitors were identified as belonging to

the dictyodendrin family of alkaloids. Cano *et al*. [79] stated the inhibition of telomerase activity by compounds derived from extracts of *Trypanosoma brucei*, *Leishmania major*, and *Leishmania tarentolae*.

In stem cells, the effect of telomerase inhibitors is minor due to the irregular proliferation of the telomerase-competent cell, and as they have much longer telomeres than cancer cells. Recent clinical experimental studies on telomerase inhibition with gene therapy have included; nucleotides and nucleoside-type reverse transcriptase inhibitors; oligonucleotide inhibitors of telomerase activity; direct inhibition by non-nucleoside small molecules; telomerase-specific phosphorylation inhibitors; G quadruplex stabilizers; and TER directed hammerhead ribozymes. The natural inhibitors of telomerase are depicted in Table 1.

Table 1. List of phytochemicals that are natural telomerase inhibitors and the effects of those verified on several cell lines and the possible mechanisms of action.

Phytochemical acting as natural Telomerase inhibitors	Type of cancer that has been tested	Cell lines used in the experimental reports	Possible mechanism of regulation
Allicin (Garlic)	Gastric	SGC-7901 [80]	Not determined
Curcumin (Turmeric)	Breast	MCF-7	Transcriptional
	Cervical	HeLa, SiHa, Ca Ski	Translational
	Gastric	SGC-7901 [81]	Post translational–Nuclear Localization
	Liver	Bel 7402 [76]	
	Leukemia	HL60 [82], K-562 [83]	
	Lung	H1299, A549	
	Brain	U87-MG, 1321N1	
Epigallocatechin Gallate (Green Tea)	Breast	MCF-7, MDA-MB-231	Transcriptional–Epigenetics Translational
	Cervical	OMC-4, TMCC-1	
	Head and Neck	Hep-2	
	Leukemia	HL60	
	Lung	H69, H69VP [78]	

3.9 Telomerase and cancer stem cell

Short telomeres were reported as causing stem-cell failure [52], whereas over-expression of the catalytic subunit of telomerase, the hTERT, could promote stem cell mobilization. Stem cell biology is well understood from the study of hematological malignancies in cancer. While telomere length maintenance in primitive human hematopoietic cells is dissociated from the activity of telomerase,

telomerase-dependent telomere shortening is found to be associated with the chromosomal instability and conversion of hematopoietic stem cells into leukemic stem cells [83]. In spite of the presence of telomerase in normal stem cells, cancer stem cells arising from the latter need greater quantities of telomerase that have great efficiency in maintaining the telomere. Henceforth, cancer stem cells can be more vulnerable to the loss of functional telomerase by telomere uncapping, therefore paving the way to hopeful therapeutic targets.

The hTERC inhibitors TTA RHPS4 and GRN163L were found to be powerful inhibitors of clonogenic tumor cell growth, i.e. colony formation. The inhibitory properties of stem cells were converted into *in vivo* antitumor activity in GRN163L and the TTA RHPS4 [83]. The RHPS4 was 1-2 log-folds more active against clonogenic tumor cells than bulk tumor mass and normal adult stem cells [84]. Cancer stem cells can be targeted with TTA (telomere targeting agents). Similar records were found in the study of an hTERT vaccine which was devised for the evaluation of hTERT vaccination effects on hematopoietic stem cells. In patients receiving the vaccine, no considerable decline was observed in macrophage, granulocyte, and erythroid colony-forming units' frequency inside bone marrow. In the case of severe combined immunodeficient or non-obese diabetic mouse repopulation assays, there was an easy detection in human hematopoietic reconstitution without qualitative or quantitative contrast in pre-and post-vaccine samples [85, 86]. From recent observation and findings, it was found that hTERT is a 'stemness' gene whereas cancer stem cell targeting indicates that tumor stem cells are differentially targeted by telomerase inhibitors.

4. *TERTp* Mutations

The mutations in the promoter region of *TERT* are termed *TERT* promoter mutations. *TERTp* mutations were discovered in melanoma in 2013 [86]. These are somatic C to T point mutations which are present at the 1,295,228 and 1,295,250 base positions found in chromosome 5. These mutations enhance the *TERT* expression (~2–6fold increase) when quantified through qRT-PCR [87]. Most solid cancers are associated with TERT promoter mutations. These mutations are found in cells that have the least self-renewal ability like brain cells, and thyroid cells. They are also found to be present in cancers related to hematopoietic cells, lymphoid tissues, and the gastrointestinal tract.

4.1 *TERTp* mutation and gliomas and glioblastoma

TERT promoter mutations are associated with increased age, prognosis, and overall survival of patients with glioma. The prognosis for patients with these mutations along with isocitrate dehydrogenase mutation is better than the patients with TERT promoter mutations alone.

4.2 *TERTp* mutation and skin carcinoma

TERT promoter mutations are highly prevalent in basal cell carcinomas (46.2%) and are associated with UV-induced skin cancers. They are associated with both melanoma and non-melanoma cancers.

4.3 *TERTp* mutation and thyroid cancer

TERT promoter mutations are often observed in follicular-cell-derived thyroid malignancies. These mutations are associated with tumor size, tumor stage, tumor recurrence, shorter survival rate, and metastasis. The outcome of radioiodine therapy is compromised by these types of mutations [88].

Apart from *TERTp* mutations, it has been reported that TERT independent activation and co-regulation of the b-catenin signaling pathways NF-kB might also be a crucial mechanistic basis for the noncanonical functions of TERT, which impart its ability to regulate the hallmarks of cancer. High levels of inflammatory cytokines such as IL-8, IL-6, TNF-alpha and several genes in the glycolytic pathway also influence TERT activity, thereby affecting the epithelial–mesenchymal transition of cancer cells and increasing their invasion and migration potentials.

4.4 Telomerase activity and assessing telomere length

Since telomerase is seen to be overexpressed in most cancers, it follows that the telomerase activity should be a promising biomarker of cancer. Several techniques have been used to measure the telomerase activity like colorimetric assays, chemiluminescent assay, PCR based telomeric repeat amplification protocol (TRAP) assays and fluorescent assays that involve isothermal amplification for fluorescent detection of telomerase activity for better sensitivity [89]. The sensitivity remains one of the critical criteria for evaluating telomerase activity since the telomerase molecule is at low concentration even in telomerase-positive tumor cells. Although *in vivo* assay for telomerase detection remains a challenge, in a complex cellular environment, efforts should be put in enhancing selectivity and high sensitivity. Additionally, telomere length (TL) measurement methods portray the average or relative TL. Incidentally, the shortest telomeres lead to telomere dysfunction and limit cell proliferation in the absence of a telomere maintenance mechanism and TL shortening is a gradual process that increases with age in humans. TRF is the most common method for research studies for TL determination but requires a lot of DNA and is labor intensive [90, 91].

5. Conclusions

This review article mainly focused on the activation of telomerase in cells that are undergoing aging and in stem cells. It was also focused on the inhibition of telomerase activity in cancer cells. Telomerase enzyme activity can be enhanced or inhibited by using active components from plant extracts and some synthetic drug formulations. Telomeres are made of single-stranded 3'overhang at the distal end and shelterin complex. Each protein subunit in the shelterin complex has a significant role in telomerase activities including recruitment to the telomere, processivity, and addition of repeats at the telomeric DNA sequence. Telomeres have a major role in capping and protecting the chromosomal ends to prevent chromosomal fusion and recombination, which may lead to severe genetic aberrations. To perform these roles, telomeres utilize telomerase enzymes to maintain lengthy repeated G sequences, and they require telomeric-associated proteins to prevent them from being recognized as a double-strand break by repair machinery. Telomere shortening is one of the major causes of aging and aging related disorders. Cellular senescence gets critically accelerated when telomeres shorten due to the presence of mutations in the telomere complex. Telomerase comprises hTR, hTRT and telomerase-associated proteins. The reverse transcriptase enzyme of telomerase has various domains and motifs to maintain telomerase structure and function appropriately. Telomerase recruitment with the telomeres is facilitated by the ACD-POT1 complex, the TIN2 subunit and phosphorylation of ACD subunit. Soon after the recruitment of telomerase with the telomere, it forms a substrate for the annealing of the telomerase's complementary RNA sequence which is serving as a template. Once the DNA/RNA hybrid forms, telomerase adds repeats to the telomeric DNA and repositions itself further to add sequences simultaneously. This is known as repeat addition processivity. Termination of telomerase action is initiated and facilitated by the CST complex and the G-quadruplex structure. Cycloastragenol is an aglycone of astragaloside IV that was isolated and formulated from the extracts of *Astragalus membranaceus*, and is recognized

as an effective telomerase activator in neuronal cells in mice. Extract of *Ginkgo biloba* also proved to be an effective activator of telomerase in endothelial progenitor cells. Gene therapy can also be performed to repair the telomerase RNA sequence and other telomerase-associated proteins. Natural telomerase inhibitors are available in plant extracts and in some marine sources, which are used for cancer treatments. Inhibitors from various natural sources are specific for certain types of cancer, and examples include garlic extract or garlic for gastric cancer, and curcumin for breast and cervical cancer. Further studies need to be undertaken to elucidate the detailed mechanism of telomeric DNA elongation and termination of telomerase activity. This will be helpful to understand the exact activity of telomerase in cancer stem cells, cancer cells and stem cells, and to design a target for therapies. This will also pave the way for the production of synthetic or natural drugs from organic sources to halt or stimulate the telomerase activity whenever it is required.

Telomerase reactivation or upregulation is a notable characteristic in 90% of cancer types. However, the biochemical mechanisms regulating human TERT expression in cancer and tumors are not completely known. The mechanisms governing the hTERT gene expression and transcription, translational and post-translational modifications must be deeply studied to know how human TERT is activated in cancer cells and its role in further disease progression and malignancy. It will be interesting to know whether the mutations in hTERT promoter and other mutations occur during the period in which cells are undergoing the growth arrest phase during the cell cycle for further oncogenic studies. Further studies and experiments on telomere-telomerase dynamics and their interaction with the genetic changes in cancer cells, stem cells, cancer stem cells, and even other cells can play an important role in the development of efficient treatments for age-related cancers and disease. Such research should also help with the design of stem cell therapies and regenerative medicine. Proper attempts should be made to narrow down the specific parts of the plant that contain the bioactive compound which plays a role in telomerase inhibition and activation. This research knowledge is not only applicable to plant compounds but also applicable to synthetically derived components and drugs. A great focus on various herbs and their bioactive components to control the reduction of telomere length, and oxidative stress in delaying the senescence in stem cells must be explored to facilitate oncogenic therapies with natural products and thus aid in the reduction of dependency of cancer treatments on radiation and chemotherapies. Efforts to expedite stem cell research and cancer studies and regenerative medicine can be translated from bench to bedside. This will involve the interdisciplinary collaboration of academics, clinical environment science, biotechnology-related fields, marketing studies, and patient experience. Molecular docking is a very good molecular simulation for basic studies in exploring the mechanisms of activity, toxicity, and the side effects of drugs by using software tools and can be used before wet-lab procedures [92]. A pivotal step in translating research into clinical therapy by developing telomere-targeted drugs, therapies and techniques is a thorough analysis of side effects, and the variation studies among different races, genetic populations, genders, habitants, and age groups [93]. One major and notable challenge of telomere and telomerase related research is the accurate measurement of telomeric region length and activity of telomerase. A combined analysis of telomere length with telomerase activity may facilitate great exhaustive clinical information compared to measurement of only a single parameter. In addition, the telomere position effect mechanism can be applied to regulate genes adjacent to human telomeres in genetic engineering techniques. Further studies on these aspects aim in throwing light on the treatment and diagnosis of many human ailments that are linked to dysfunction of telomerase enzymes and the silencing of telomeres. The dynamic features of telomerase and telomeres make their structural and biochemical studies difficult. Hence single-molecule approaches facilitate feasible and effective studies of telomerase and telomere components functioning. Single-molecule methods facilitate an opportunity to know more about the complex dynamics of telomerase and telomeres by allowing researchers and scientists to visualize and manipulate the RNA, DNA, and individual proteins necessary for the normal telomere function.

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