

Chapter 10

Telomerase and its Inhibitor in Cancer Therapeutics: Current Status and Future Prospective



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Abstract Telomerase is an extremely specific reverse transcriptase (RT) that involves maintaining telomeric length by duplicating TTAGGG nucleotide DNA sequences on the human chromosomes at the 3' terminal position. It functions as a shielding effect from degradation and loss of gene sequences. In humans, the ribonucleoprotein complex consists of a catalytic subunit: hTERT (human telomerase reverse transcriptase) and RNA subunit: hTR (human telomerase RNA). It is expressed in embryonic cells and suppressed during maturity. The enzyme is reactivated in around 85–90% of solid tumors. These findings mark it a likely drug target that could be established for therapeutic purposes with negligible effects. Herewith, we assess recent approaches to telomerase-directed therapy, deliberate the aids, their shortcomings, and speculate on the forthcoming perspective of inhibitors that target telomerase as cancer therapeutics.

Keywords Telomerase · Telomerase activity · Cancer · Telomerase RNA · Inhibitors

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10.1 Introduction

The nucleus of a eukaryotic cell, as seen under the microscope, which is about to divide, appears as double-sausage-like structures called chromosomes. At the end of these double-sausage-like structures, we see molecular probes labeled for visualization of the DNA, called telomeric DNA, which has many tandem copies of short oligonucleotides sequences. They could vary from a few base pairs to a few thousand base pairs. These G-rich telomeric DNA sequences at the 3'OH are the unit of telomeres that function as a cap to the end of chromosomes. The sequence is T_xG_y on one strand and A_xC_y on the other strand, where x and y can range from 1 to 4. The TG strand stretches longer than its complementary strand, leaving the strand rich in "G," as a single strand of DNA, hanging up to a few hundred nucleotides towards the 3'OH end. These help in stabilizing the chromosome. The human telomeric region consists of tandem arrays of non-coding hexameric repeat sequences, 5'-(TTAGGG)n-3', which is followed by a G-rich single-stranded that overhang (150–200 nucleotides long) at 3'OH terminally (Fig. 10.1). The exact sequence of the telomeric repeat can vary from species to species (Blackburn and Szostak 1984).

Their size ranges from 15 to 20 kbp to sometimes 50 kbp in chronic disease or tumors. Telomeres get reduced with each cell cycle. They are just like the plastic end of the shoelaces. Just like the plastic ends prevent the threads from segregating and protect the shoelace from ruining telomeres, it helps maintain chromosome integrity and provides a buffer of expandable DNA (Dunn et al. 1984).

There can be two types of end in DNA: end of chromosomal DNA and DNA breaks. These DNA breaks can be repaired via different types of DNA repair mechanisms found in cells. One of the aspects of telomere is to function as caps to the tips of chromosomes to prevent telomeres from undergoing DNA reactions which could be one of the consequences of broken DNA ends. If these breaks at the end are not repaired, DNA can undergo degradation. Therefore, it is clear that the

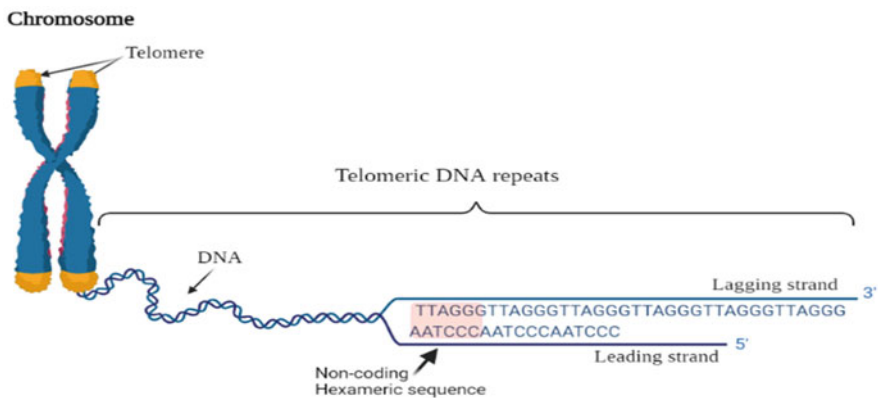


Fig. 10.1 Representing human chromosome and telomeric repeats at its end. (Source: Figure generated at BioRender (<https://biorender.com/>))

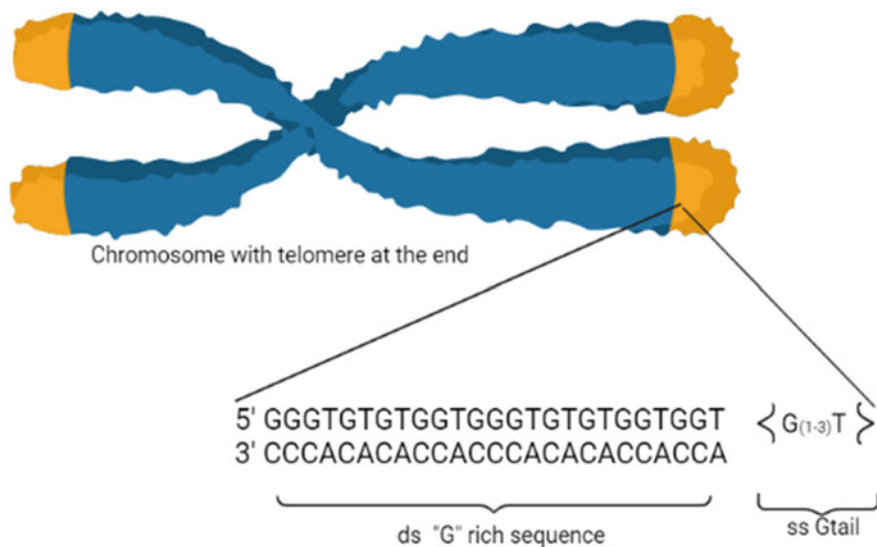


Fig. 10.2 Telomeric DNA sequence at the end of *Tetrahymena* chromosome representing the ds strand and the hanging G-tail. (Source: Figure generated at BioRender (<https://biorender.com/>))

telomeres prevent DNA from undergoing degradation. These DNA sequences at the tip of chromosomes are repetitive and similar; for example, TTAGGG is one of the short sequences repeated over again in human chromosomes. These repeats are called telomeric simple tandem repeats.

Another feature of telomeric DNA in chromosomal DNA is that it is single-stranded, unlike the remaining. The orientation of telomeric DNA is 5'–3' towards the end, as shown in Fig. 10.2 (Jafri et al. 2016).

These telomeric sequences are repetitive non-coding DNA sequences. These sequences attract specific proteins like the shelterin protein that can bind to the double- and single-stranded portions of telomeric DNA repeats (Herbert 2011). The end of the telomere is protected by a group of proteins present in variable amounts known as the Shelterin complex, and it forms a T-loop (telomeric loop) and a small D-loop (displacement loop). The 3' G-rich DNA sequence, which is overhanging, assists telomeric DNA in constituting a more advanced structure. The 3' single-strand, which is overhanging, folds backward and occupies the homologous double-stranded TTAGGG region, forming a telomeric loop (t-loop). T-loop prevents the end of telomeres from being acknowledged as breakpoints by the DNA repair mechanism. The Shelterin complex comprises three core shelterin subunits, namely telomeric repeat binding factor (TRF1 and TRF2), human protection of telomeres 1 (POT1), TERF1-interacting nuclear factor 2 (TIN2), tripeptidyl peptidase 1 (TPP1), and repressor/activation protein 1 (RAP1) (van Steensel and de Lange 1997). Each subunit of shelterin separately consists of the following:

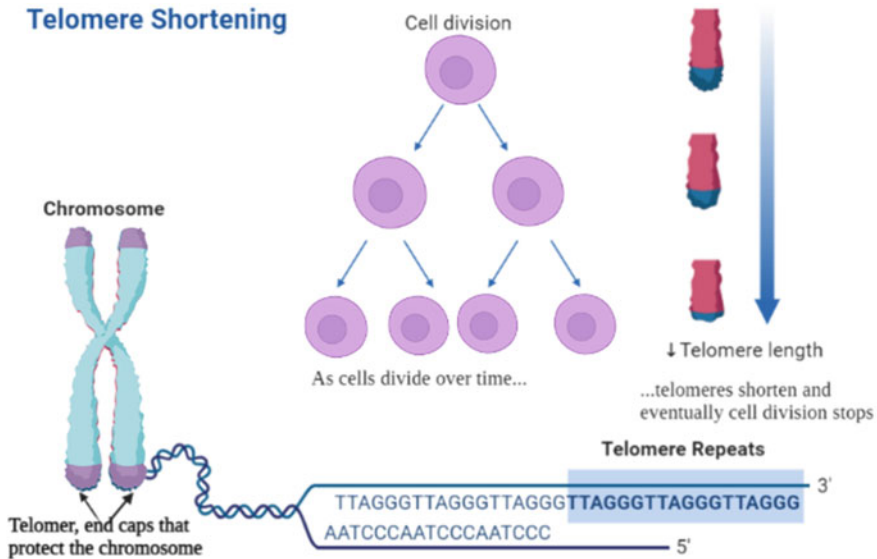


Fig. 10.3 Representing shortening of telomerase with every cell division. (Source: Figure generated at BioRender, (<https://biorender.com/>))

TRF1 (Telomeric Repeat Binding Factor 1): It is a dimeric protein of identical subunits that controls telomeric DNA replication by binding to the ds (double-stranded) TTAGGG domain of telomere. It decreases with aging in humans and mice (Takai et al. 2010).

TRF2 (Telomeric Repeat Binding Factor 2): It is anatomically related to TRF1, which is necessary for t-loop formation. It binds to the ds TTAGGG region of the telomere, overexpression of TRF2 results in shortening the telomere. Loss of TRF2 results in loss of t-loop, activating p53, and ATM-arbitrated apoptosis (Palm and de Lange 2008).

POT1 (Protection of Telomeres 1): It consists of OB folds (oligonucleotide/oligosaccharide binding). The OB folds increase POT1's affinity for the single-stranded TTAGGG region of the telomere. POT1 assists in forming a telomere stabilizing d-loop. It also prohibits ss-DNA degradation by nucleases and shields the 3'G overhang and subdues ATR-intervened DNA repair (Liu et al. 2004).

RAP1 (Repressor/Activator Protein 1): It inhibits DNA repair and is a stabilizing protein associated with TRF2. Its main function is to regulate transcription and influence NF- κ B signaling (Celli and de Lange 2005).

TIN2: It binds to TRF1, TRF2, and TPP1-POT1 complexes, stabilizes them, and bridges complexes linked to double-stranded DNA and single-stranded DNA TTAGGG region of the telomere. It also promotes glycolysis (Takai et al. 2003). Each time the cell divides, it results in the shortening of the telomere (Fig. 10.3). Cell division leads to growth arrest (replicative senescence) and gradually apoptosis. It has been reported that replicative senescence occurs

when one or more critically short telomeres trigger a DNA damage response regulated by p53. This growth arrest stage can be bypassed temporarily when p53 and RB are disabled. Nevertheless, till this time, the telomere is excessively shortened and leads to multiple chromosome end fusion that ultimately leads to loss of cell viability (Fig. 10.3) (Fagagna et al. 2003; Smith et al. 1998; Lee et al. 2006).

When there is an alteration in genomic stability, telomere maintenance is disrupted, resulting in end-to-end chromosome fusion or ends being represented as a double-stranded break. Many studies have suggested that cells respond to telomere dysfunction by undergoing apoptosis, genome instability, or senescence. There could be different reasons for telomere dysfunction, such as shortening the length of telomeric DNA, which can be caused due to malfunctioning of an enzyme called telomerase malfunctioning, and telomere dysfunction that can be caused when the proteins cannot bind to telomeric DNA. Molecular interruptions dismantle this binding of protein to telomeric DNA. Both can cause slowing of cell division and loss of cell renewal capacity leading to genomic instability. Telomere dysfunction can be sensed because they have regulatory action against the malfunctioning of DNA. End-to-end fusion of telomeres between two chromosomes can happen if the protein attached to telomeric DNA repeats is disrupted. This fusion of two chromosomes will lead to two centromeres in a chromosome. When it divides during cell division, the chromosomes will be ripped apart, leading to genetic instability (Galati et al. 2013). The machinery of DNA replication has high fidelity. However, DNA replication machinery cannot copy the end of linear DNA (in eukaryotic chromosomal DNA) that can be demonstrated. Every time DNA replicates as the cell divides, the daughter DNA gets shorter and shorter after every replication, concluding that the DNA ends cannot be replicated without compensatory loss. This compensatory loss after every replication can lead to senescence of the cell.

In the early 1970s, James Watson and Olovnikov, after seeing the machinery of DNA replication, concluded that senescence of cells after multiple cell division is due to loss of terminal DNA. Loss of terminal DNA during cell division is the reason that human cells cannot proliferate indefinitely in culture (Corey 2009). It is, therefore, essential to prevent the shortening of telomeric DNA, which will further prevent cell death (Osterhage and Friedman 2009).

10.2 Telomerase: The Anti-Aging Enzyme

In the late 1970 and early 1980s, telomerase was observed in a ciliated protozoa *Tetrahymena*, which has many small minichromosomes that help in the molecular analysis of telomeric DNA. Telomeric repetitive sequences in *Tetrahymena* (GGGGTT) were heterogeneous in different chromosomes, which means some mini chromosomes had 20 repeats of this sequence, some had 50, some had 200, and so on. At the same time, these were supposedly expected to be homogenous

in a population of *Tetrahymena*. This led to a question on the replication of telomeric DNA (Blackburn 2010). Another observation was made in a single-celled parasitic organism that causes sleeping sickness called *Trypanosoma*. It was found that telomeric DNA gradually got longer (Hayflick 1998; Blackburn and Challoner 1984). In another experiment, yeast telomeric (TG (1–3) repeats) DNA was grafted on *Tetrahymena* (TTGGGG) telomeric DNA repeats, and the linear plasmid formed was introduced into a yeast cell. It was observed that the ends of the DNA were maintained in the yeast cell as linear minichromosomes after replication. This observation was contrary to the standard known model of DNA replication or DNA recombination. All these observations suggested that a cell was capable of adding telomeric sequences (Shampay et al. 1984).

In her experiment, Barbara McClintock, a geneticist working on maize, noted that a mutant maize stock lost the capacity found in normal wild-type maize. If the chromosome breaks by radiation or mechanical rupture in wild-type maize, the broken ends of chromosomes can be healed to make a normal stable telomere. Nevertheless, she discovered this capacity of maintaining stable telomere had been lost in a mutant stock (McClintock 1941). All of these were directed towards a question then raised “if there was an enzyme that could extend the telomeric DNA sequence?” Elisabeth Blackburn and Carol Greider, in the early to mid-1980s, designed an experimental system with a high number of minichromosomes and, therefore, a high number of telomeres to study the presence of enzymes that can extend or maintain telomeres. They choose single-celled ciliated protozoan *Tetrahymena thermophila*. “G”-rich strand of telomeric DNA found at the tip of chromosomes was taken as an oligomer with a free 3'OH end. It was mixed with extract of *Tetrahymena* cell S-100 at a developmental stage at which telomeric sequence of DNA was added to freshly broken ends of chromosome because that would be very likely for an enzyme (if present) to show high activity than when present in the normal state. Mg^{2+} was added, followed by nucleotide triphosphate precursor (radiolabeled dGTP and TPP) (Fig. 10.4). This reaction mixture studied under autoradiograph found that the DNA sequence at the end of the telomere was added to the oligomer.

This experiment confirmed the assumed hypothesis that the given sets of newly added telomeric repeats were added due to the presence of an enzyme, telomerase (Greider and Blackburn 1985). Telomerase is a cellular ribonucleoprotein DNA polymerase enzyme. It is responsible for the extension and maintenance of the telomere and adds the TTAGGG sequence to the 3' end of the chromosome. Telomerase activity is necessary to overcome the shortening of telomere and increase the ability of the cell to divide limitlessly. Telomerase is a unique and interesting enzyme because it has a DNA polymerase and RNA sequence that acts as a template for synthesizing telomeric repeats of DNA. Part of the RNA sequence hybridizes with the single-stranded overhanging DNA sequence (Sedivy 2007).

Telomerase has an associated RNA with a nucleotide sequence complementary to the telomeric repeat sequence. Using complementary RNA as a template adds the nucleotides and extends the 3' overhang telomeric DNA strand. A matching or complementary synthesis of strand can be done by the standard DNA machinery,

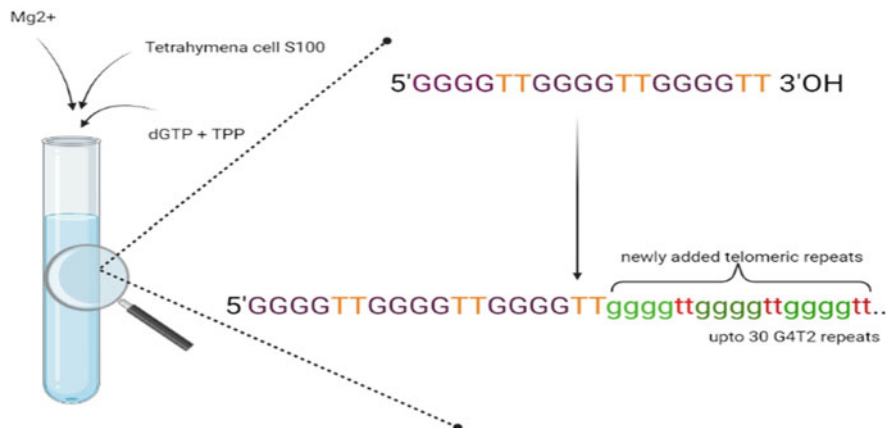


Fig. 10.4 Schematic representation of experiment carried out by Blackburn and Greider, using *Tetrahymena* cells which led to the discovery of telomerase. (Source: Figure generated at BioRender, (<https://biorender.com/>))

which uses an RNA primer and DNA polymerase, producing ds DNA when the overhang is extended long enough. These repeats were added to the DNA strand corresponding to the sequence at the 3'OH end of a chromosome and found that the complementary strand was not competent to add telomeric repeats (Bodnar et al. 1998).

In another experiment, yeast telomeric repeat with different sequences (TGTGTTGGTGGGT ...) was used as a primer oligonucleotide to *Tetrahymena* extract in a similar reaction mixture. It was found that the repeats added by telomerase (enzyme) maintained the alignment of the addition of a new sequence of telomeric repeats to maintain the set of G₄T₂ depending on the 3'OH of the primer (Greider and Blackburn 1985).

This concludes that there could be a template within the telomerase enzyme complementary to the oligomer that maintains the G₄T₂ sequence when *Tetrahymena* cells are added to yeast telomeric oligomer repeats (Dunn et al. 1984) (Fig. 10.5). Later, a built-in template was found to be present in the telomerase complex, which was made up of RNA. This template is a short portion of RNA (human telomerase RNA) with other subunits like a protein called human telomerase reverse transcriptase (hTERT) that has reverse transcriptase activity. The telomerase enzyme complex consists of two significant subunits: a catalytic subunit, hTERT, which has reverse transcriptase activity, and a structural RNA component, hTERC or hTR.

This provides a template of 11 bp to encode telomeric repeats that are to be added to the chromosome. The expression of the human telomerase RNA gene (hTERC) occurs in both normal and cancerous cells, while hTERT expression is limited to a cancerous cell. Telomerase activity and hTERT mRNA expression are linked to human cancers. Experiments have shown that hTERC levels are unregulated in the early neoplastic stages, which further increases during the progression of tumors.

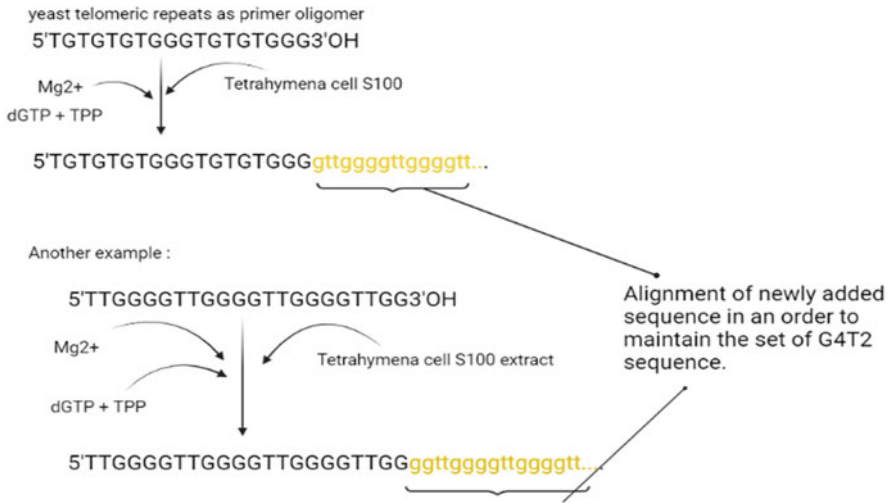


Fig. 10.5 The experimental system shows how the newly added sequences are aligned to maintain the G₄T₂ sequence due to the template RNA component in telomerase. (Source: Figure generated at BioRender, (<https://biorender.com/>))

Whereas regulation of telomerase activity is primarily by hTERT and is detected only in the late stage of tumors. This indicates that the regulation of telomerase activity is different from the expression of its RNA component. Initial upregulation of telomerase RNA is responsible for the hyper-proliferation of cancer cells. Therefore, telomerase activation confirms the unlimited proliferative capacity of the emerging and evolving cancer cells. However, telomerase activity is not correlated with tumor invasion. Telomerase activity is a decent marker for detecting gastric carcinoma (Blackburn 2001; de Lange 2005).

10.3 Mechanism of Action of Telomerase

Telomerase takes a single-strand rich in G sequence and aligns the 3'OH end of the ss-strand by Watson-Crick base pairing rule onto the RNA template sequence present in the telomerase enzyme. Once aligned, it polymerizes complementary nucleotides, extending the telomeric DNA. This proves that telomerase is a unique polymerase, and it copies RNA to DNA hence also a reverse transcriptase. It has an intrinsic RNA component that helps synthesize short repeats on the telomeric end of chromosomes (Fig. 10.6). *Tetrahymena*, when grown in the culture they keep on propagating. In other terms, they are effectively immortal. One of the reasons could be the presence of telomerase in high amounts. By manipulating telomerase in *Tetrahymena* (where it was initially discovered), it can lead cells towards senescence. Manipulation can also be done by changing a few nucleotides of its RNA

telomeric DNA sequence, which activates the DDR pathway specific to the telomere. This pathway stimulates the synthesis of DNA sequences at the end of the telomere. Flynn and his colleagues discovered that the ALT mechanism could be disrupted by protein kinase ATR inhibition in all cells that have the ALT mechanism. That will result in the death of the cell. Hence, ATR inhibitors can be considered therapy to all the tumors resulting from the ALT mechanism (Flynn et al. 2015).

Cells multiply, and their telomeres will subsequently become shorter in the absence of telomerase. To counteract the shortening of telomeres, eventually, cells will cease to divide. Cells respond to short telomeres, also called senescence response, in which cells will not replicate DNA anymore, which ultimately will lead to cell death. One can propose that apoptosis can be induced by malfunctioning telomerase. Without it, human cells can lose telomeric DNA. Human telomeres are made up of thousands of copies of telomeric repeats. So it would take a lot of cell cycle of cell division before the cell dies. If telomerase catalytic function is inhibited, it will not carry out DNA polymerase reaction by reverse transcriptase, causing the cell to die eventually. Hence, the proliferation of tumor cells can be stopped. When the catalytic function is inhibited, the ribonucleoprotein level of the enzyme “telomerase” is kept high because the purpose is not to deplete the cell of the enzyme but to render the enzyme inactive. By knocking down telomerase RNA, there was inhibition of cancer cells. Human telomerase can be knocked down using RNA-hairpin-SiRNA (Short interfering RNA). Two strands of RNA complementary to target RNA cause the breaking down of target RNA (Blackburn et al. 2006).

Telomerase knocked down in cancerous cells causes a reduction in metastasis by downregulating the cell cycle and tumor progression genes. It also downregulates glucose metabolism as cancer cells have high glucose metabolism. The replicative capacity of fibroblasts cells can be predicted by studying their telomeric length. Loss of telomeric DNA repeats induces signals that regulate cell division and apoptosis of the cells.

10.4 Telomerase: A Critical Hallmark of Cancer

Cancer is one of the most aggressive pathological disorders in humans all over the world, some of its types are curable, and some have lasted for a long time. Cancer could be considered an age-related disorder. Many reasons and factors provide a favorable environment for cancer cells to develop and spread. Moreover, this enzyme is one of those factors which are responsible for causing cancer in humans. Telomere is the non-coding repetitive sequence, present at the end tip of chromosomes, maintains stability, and protects human chromosomes (just like the plastic thing, present at the end tip of shoelaces). However, every successive cell division in the body system would shorten the telomere length and, this process limits the proliferative ability of cells to a certain number of cell divisions by inducing cellular senescence or apoptosis.

So, the mechanism of maintaining the length of chromosomes (telomere length) ever after the cell division is done by telomerase which adds the guanine-rich repeated nucleotide sequence at the end of the chromosome to regain its actual length after the successive cell division. Telomerase is predominantly seen in human cancer cells, about 85–90%, not in the normal cell of humans. The re-expression and reactivation of the telomerase enzyme are responsible for uncontrolled growth of the cell, survival of tumors, and tumorigenesis. Therefore, telomerase is also known as “immortal enzyme” (Bourgoin 2012).

10.5 Identification of Cancer Cells

Since cancer cells contain telomerase enzymes in very high concentrations, it is used as a key to identifying these abnormal cells. Based on the level of telomerase enzyme in the cells, the behavior of tumor cells (benign or metastatic) could also be determined easily. According to molecular data, very high telomerase activity is found in breast, gastrointestinal, and colorectal cancer patients. Only 15% of cancers do not express telomerase enzymes. Therefore, nowadays, telomerase is one of the recommended diagnostic biomarkers for different types of cancer treatment and diagnosis (Sarvesvaran 1999; Nakanishi et al. 2002; Oztas et al. 2016). Scientists have been investigating telomerase as a predominant tool for cancer diagnosis and treatment. It is a reliable marker for developing new cancer therapies; hence, deactivation, destabilization, and suppression are key to curing cancer. What are the factors that activate the telomerase enzyme so many times in abnormal cells? What genes or what specific nucleotide sequence is responsible for it being investigated, enhancing information for anti-cancer drug design? Strategies that inhibit telomerase’s action or function in the cancer cell include many drugs, SMI (small molecular inhibitors), vaccines, etc. Telomerase inhibitors as anti-cancer agents could be one of the most reasonable and reliable strategies (Huang et al. 2013).

10.6 Telomerase Inhibitors

Eukaryotic telomerase contains reverse transcriptase components (hTERT), a catalytic protein subunit, and RNA components (hTR), essential for adding repetitive sequences at the ends. Researchers have telomerase inhibitors that contain RNA and RNA binding protein parts for better binding affinity (Kazemi-Lomedasht et al. 2013). Telomerase inhibitors are derived from natural sources as well as synthetically generated. Moreover, some inhibitors contain modified oligonucleotides. Telomerase binding agents like G4 ligands (quadruplex ligands) and ALT cells play a primary role in inhibitions. Stabilizations by G4 ligands and deactivation of telomerase or telomerase gene suppression are new opportunities to target cancer cells and tumors. Usage of synthetic inhibitors against telomerase shows some side effects and

complications in cancer patients. On the other hand, natural inhibitors against telomerase have very few or no side effects. Natural inhibitors, taken from the diet, are better suppressors and healers and are safe to consume than synthetic inhibitors (Chen et al. 2011; Badrzadeh et al. 2014; Zhang and Wang 2017).

10.6.1 AZT: Inhibitor of Reverse Transcriptase

2'-Azido-2' 3'-dideoxythymidine (AZT), an analog of thymidine, has the ability to inhibit reverse transcriptase. It is an antiviral agent that is also used to treat HIV/AIDS, as it inhibits the replication of HIV by blocking its reverse transcriptase. When it is phosphorylated by thymidine kinase, it forms AZT-TP (Falchetti et al. 2004), which can be incorporated at the place of thymidine in DNA. It has a low affinity towards DNA polymerase alpha, beta, gamma but a high affinity towards reverse transcriptase (Faraj et al. 2000). AZT integrates with CHO DNA cell line (most commonly used mammalian cell line, derived from Chinese hamster ovary) with the help of immune fluorescence tagged antibodies produced against AZT. After incorporation, followed by separating telomeric DNA from genomic DNA via methods like restriction digestion and size fractionation of DNA to quantitatively compare the incorporation of (H^3)-AZT in telomeric and non-telomeric regions of CHO cell lines (Olivero and Poirier 1993), it was found that (H^3)-AZT integration was three times more in the telomeric region than in the non-telomeric region (Gomez et al. 1995). Cells of dermal fibroblasts of mice have long telomeric repeats, but they do not have telomerase, and it was seen that they did not incorporate (H^3)-AZT in their telomeric region.

Incorporation of (H^3)-AZT in the telomeric region of CHO cell lines with telomerase, while it did not get incorporated in cells of dermal fibroblasts of a mouse that did not have telomerase, showed it regulates AZT incorporation. Later it was experimentally proven that AZT either inhibits telomerase or shortens its length. Strahl and Blackburn in 1996 found shortening of telomerase in B cell line JY616 and T cell line JukratE61 when passed through 100 micromolar AZT, which confirms that AZT inhibits telomerase (Strahl and Blackburn 1996). Yegorov et al., in 1996, confirmed induction of apoptosis in the culture of immortal fibroblast of a mouse with the help of AZT. This process was reversible as after removing inhibitors (AZT), the cell entered the cell cycle again. They concluded that AZT blocks telomerase function in mouse cells (Yegorov et al. 1996).

Later on, it was found that AZT inhibited the progression of all tumor cell lines. For example, Multani in 1998 conducted an experiment using fluorescence in situ hybridization, discovered a reduction in telomeric length in murine melanoma (K-1275 clone X-21) and human breast cancer cell line (MCF-7) when treated with AZT. Human endometrial carcinoma cells (HEC-1) showed similar results. The reduction in length of telomeres can be seen in HeLa cells after exposure to AZT for an extended period without senescence. Concluding, telomeric inhibition by AZT depends on the concentration of AZT and duration of exposure. Sometimes

AZT exposure might not lead to senescence or cell death because AZT-resistant phenotype could have been developed. The number of AZT treatment passages is insufficient for a cell to go to senescence, or a different mechanism called alternative lengthening of telomeres is compensating for the telomeric loss (Multani et al. 1998).

Telomerase subunit hTERT and c-Myc's activity reduced on treatment with AZT followed by alteration in hTR, Mad1, hTERT. AZT also causes a reduction in checkpoint kinases (Chk1) and (Chk2) and an increase in phosphorylated Chk1 (Ser345) and Chk2 (Thr68). Telomerase inhibition will not affect humans until telomeres reach a critical size, causing the cell to die. This means during telomerase inhibition, tumor cells will continue to grow for some divisions until telomeres inside these cells reach a critical size leading to cell death (Gomez et al. 2012; Hájek et al. 2005).

10.6.2 Natural Telomerase Inhibitors (NTI)

Natural telomerase inhibitors (NTI) are derived from natural sources such as plant material and include secondary metabolites like alkaloids, polyphenols, triterpenes, xanthenes, indol-3-carbinol, telomestatin, gingerol, etc. (Ganesan and Xu 2017). The alkaloid inhibitors include Boldine (natural aporphine alkaloids) and Berberine (isoquinoline quaternary alkaloid). Another category of natural telomerase inhibitors is polyphenol inhibitors such as curcumin, quercetin, and resveratrol (Chen et al. 2011). Table 10.1 summarizes naturally derived telomerase inhibitors with structures, properties, probable mode of action.

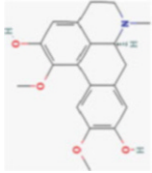
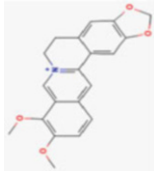
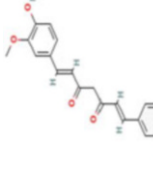
10.6.2.1 Inhibitors Targeting hTERT



2-((E)-3-naphthalen-2-yl-but-2-enoylamino)-benzoic acid (BIBR1532) is a small synthetic non-nucleic and non-peptide compound, which links to the hTERT in its active site, competitively inhibiting telomerase. It is the most promising hTERT inhibitor among the inhibitors developed to date. BIBR1532 binds to the reverse transcriptase active site of hTERT non-covalently and inhibits telomerase, which reduces the number of added TTAGGG repeats. BIBR1532 does not cause chain termination. It can act as translocating enzyme-DNA-substrate complex or favoring the DNA substrate disjunction from the enzyme during the copy of the template (Pascolo et al. 2002).

10.6.2.2 hTERT Immunotherapy

The property of cancerous cells to overexpress their efficient, functional telomerase, and specific epitopes against hTERT helps in targeting the telomerase. Therefore, they can be eliminated by immune system stimulation with specific vaccines.

Table 10.1 Natural telomerase inhibitors, properties, structure, and mode of action

Inhibitors	Derived from	Properties	Mode of action	Structure	Reference
Boldine (an alkaloid)	Boldo plants (<i>Peumus boldus</i>)	Anti-inflammatory and antioxidant properties It was found to inhibit telomerase function using (q-TRAP quantitative real-time telomerase repeat amplification protocol). It also reduces cell viability and cell proliferation in T24 cells Majorly used to treat breast cancer (MCF-7) and MDA-MB-231 cells and embryogenic kidney (HEK293)	Plays a role in changing the splicing variant of hTERT Blocks synthesis of prostaglandin by inhibiting cyclooxygenase activity and reduces the effect of inflammatory cytokines and cell proliferation		Kazemi Noureini and Tanavar (2015)
Berberine (an isoquinoline quaternary alkaloid)	Root, stem, bark of European barberry (<i>Berberis vulgaris</i>), and turmeric (<i>Curcuma longa</i>)	They are used for the treatment of cancer, diabetes, heart failure, high blood pressure, <i>H. pyloric</i> infection It can also bind with the G-quadruplex structure of DNA	It inhibits telomerase activity by stabilizing G-quadruplex It also inhibits the AMPK pathway Responsible for weakening the strength of cancer cells		Wu et al. (1999)
Curcumin (a polyphenolic)	Turmeric plants (<i>Curcuma longa</i> L.)	It has various antitumor, anticarcinogenic, and antiinflammatory properties It is considered ineffective in brain tumor cells and binds to the cell surface to provoke apoptosis It plays a vital role in cell viability	It was found to inhibit telomerase activity in MCF-7 breast cancer cells Curcumin increases the production of ROS and inhibits special protein 1 binding affinity, followed by downregulation of hTERT		Chakraborty et al. (2007); Ramachandran et al. 2002; Lee and Chung 2010)

<p>Quercetin (a polyphenolic)</p>	<p>Flavonoid (secondary metabolites found in plants)</p>	<p>It is highly recommended in adjunct cancer therapy It is considered a primary chemoprevention agent in several cancers such as prostate, breast, lungs, colon, etc. It has anti-inflammatory, free radical oxidant, and antitumor activities</p>	<p>Inhibitor of PI3K and NF-K3 It also inhibits cell proliferation and cell transduction in cancer cells</p>		<p>Choi et al. (2001), Kuo et al. (2004); Ayşe et al. (2011)</p>
<p>Resveratrol (a phenolic phytoalexin)</p>	<p>Found in various plants and in the skin of fruit like peanuts, grapes, etc.</p>	<p>It has chemopreventive potential Its anticancer property shows direct inhibitory action as well as cell proliferation, apoptosis, and signal transduction Pterostilbene reduces the catalytic action of telomerase It is most effective in reducing cell viability</p>	<p>Telomerase inhibition activity and downregulation of hTERT protein expression in cancer cell lines Treatment with pterostilbene (as an analog of resveratrol) decreased telomerase activity and protein expression in lung cancer line H460 It also inhibits the cell proliferation of HT-29 and WDr cell line and downregulation of telomerase activity in a dose-dependent manner Inhibits promoter activity of hTERT and prevents cell proliferation in colon cancer</p>		<p>Chen et al. (2017)</p>

Targeting telomerase for immunotherapy stimulates immune cells against tumors expressing hTERT peptides as surface antigens. As a result, telomerase-specific CD8⁺ cytotoxic T-lymphocyte amplification is regulated to target and exterminate telomerase-positive tumor cells. Numerous vaccine strategies have been developed and used in recent times, including hTERT-specific immune responses (Ellingsen et al. 2021). Some of them include GV1001, Vx-001, 1540. Tumor-associated antigens are self-proteins in humans, and their precise T-cells are tolerated. Self-tolerance is commonly regulated against “dominant” (high affinity for HLA) but not against “cryptic” (low affinity for HLA) peptides. Hence, the easiest way to evade this self-tolerance is to use cryptic peptides as in Vx-001. In cancer immunotherapy, overwhelming this tumor-specific self-tolerance is a primary goal (Jafri et al. 2016; Gellert et al. 2005; Greider and Blackburn 1985; Incles et al. 2003).

10.6.2.3 Antisense against hTR and hTERT

The catalytic activity of telomerase needs an intrinsic RNA template. The antisense regulated against the template region of hTR (5'-CUAACCCUAA) tends to be both selective and potent. In 1994, it was demonstrated that telomerase activity could be inhibited by treating cells with appropriate antisense molecules. However, the problem that arises is the stability and uptake of antisense when attempting treatment with oligonucleotides. Adding the 2'-*O*-(2-methoxyethyl) (2'-MEO) group to the antisense RNA molecule targets the 11 base pair template region inhibiting the telomerase. The antisense molecules against both hTR and hTERT form a duplex with the targeted mRNA and prevent translation of the target protein (Mender et al. 2015a, b).

10.6.3 *Altering Telomerase Activity to Induce Telomeric Dysfunction, which Causes Cancer Cell Death*

A long lag period to observe telomerase-induced cell death by anti-telomerase-directed therapy is a significant challenge we are facing today. It requires a series of cell cycles and cell division to induce relevant effects in therapeutically reducing tumors. In this phase of treatment, most of the tumor cells will grow, further requiring other treatments for a clinical outcome. Natural telomerase inhibition therapy is given to patients with other toxicities, such as hematological toxicities, which require drug holidays. Stopping treatment for a few days will reverse the benefit of therapy. Therefore, it is desirable to use therapeutic agents that act fast, which can inhibit telomerase activity.

One of the methods of doing so is not directly targeting telomerase; instead can be done by introducing modified nucleoside into the cell, which might help incorporate telomerase into telomeric DNA. When this nucleotide is incorporated into the

telomere, it will not bind to the Shelterin protein effectively, leading to the rapid death of the cell.

Mender and colleagues proved that a telomerase-positive cell was introduced to a nucleoside analogous to 6'-thioguanine (6'-thio-2'-deoxyguanosine). It got incorporated into telomeres, resulting in telomeric dysfunction as the telomeric-associated DNA damage signal was activated and led to cell death. This treatment to cell lines showed a significant reduction in tumor growth rate. Hence, it was concluded that telomere disruption mediated by telomerase could be a window to effective cancer treatment (Mender et al. 2015a, b; Corey 2002).

10.6.4 Antagonist Template to hTR (RNA Template of Telomerase)

It delivers oligonucleotides complementary to the template RNA component (hTR) of telomerase into a tumor cell. It has its advantages and disadvantages. One of the significant disadvantages is that inhibiting telomerase takes longer to show telomere's shortening effect in tumor cells. Oligonucleotides targeting the RNA component of telomerase serve as a classic enzyme to inhibit telomerase activity. In some experiments, the antagonistic oligonucleotides administered into tumor cells showed a reduction in telomerase activity and telomerase activity, which leads to telomere shortening and decreases in cell proliferation, and on the other hand, it increases apoptosis rate (Shay and Wright 2002).

It was observed that when this antagonist template was removed from the culture, the telomerase activity of cells was regained and entered the cell cycle again, growing their telomeres back. This observation revealed that the action mechanism is based on competitive inhibition of the enzyme telomerase (Corey 2002; Herbert et al. 1999; Lee et al. 2001).

10.6.5 Combination Therapies

A pitfall of the telomerase inhibitor is the lag time, and it typically requires shortening the telomere length and the cell to respond by arresting its growth, which we can overcome by the combination of inhibitors of telomerase with existing chemotherapeutics. When combined with telomerase inhibitors via antisense, DNA damaging drugs like doxorubicin and daunorubicin create a knockout hTR or inactivate the hTERT, respectively, increasing the sensitivity of cells towards DNA interacting drugs. Because telomerase has a role in DNA repair and replication, if components of telomerase are knocked out, it will render the cell's ability to protect it from DNA damage (Nakajima et al. 2003; Ishibashi and Lippard 1998).

10.6.6 Cisplatin in Cancer Therapy

Cisplatin, or cis-diamminedichloroplatinum II, is a well-known platinum-based chemotherapeutic drug/agent. It has been introduced to treat human cancer such as testicular, ovarian, breast, brain, lungs, liver, neck, etc. M. Peyron first synthesized it in 1844. Also, its first FDA-approved platinum component for cancer treatment was in 1978. For the first time, Burger et al. showed that cisplatin minimized telomerase activity in tumor cells like testicular tumor cells (Burger et al. 1997; Greider 1998). Molecular results showed that cisplatin also affects the gene transcription and reduction in the expression of hTR in testicular tumors. Cisplatin is a crucial ingredient in the systematic treatment of germ cell cancer. Ishibashi and Lippard (1998) first discovered the loss of telomere in HeLa cells after treating it with cisplatin.

10.6.7 Mode of Action

Cisplatin is a chemotherapeutic drug that can direct interactions with DNA and shows crosslinking with purine bases in the ds DNA. It inhibits DNA synthesis, has cytotoxic activities, causes DNA damage, and promotes apoptosis in abnormal cells. Cisplatin can also reduce telomerase activity and the length of telomere in the tumor cell. It could also induce apoptosis and arrest the cell cycle at specific stages, and it could induce oxidative stress, which leads to the production of reactive oxygen species (ROS). It could also induce p53 tumor suppressor signal transduction and downregulation of proto-oncogenes. High doses of cisplatin have various taxological effects such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and other organ toxicity. Cisplatin is an antitumor chemotherapeutic agent/drug used in combination with other drugs such as paclitaxel, doxorubicin, UFT, gemcitabine, osthol, etc. According to several studies at the molecular level, combining cisplatin with antitumor other medicines is the best therapeutic approach to reduce cisplatin's toxicity or toxic effect and reduce the inflammatory effects in cancer patients (Nakamura et al. 1997).

10.6.8 Cisplatin and Primary Hepatocellular Carcinoma (PHCC)

A new strategy has been introduced to treat the solid tumor, primary hepatocellular carcinoma (PHCC). PHCC is one of the most aggressive and primary types of liver cancer, which causes several deaths per year worldwide.

Cisplatin affects the telomerase activity in hepatic cells because PHCC has a high concentration of telomerase enzymes, leading to unlimited liver cell proliferation.

Telomerase activity in liver cells is clinically analyzed by PCR based on TRAP (telomeric repeat amplification protocol), and the rate of apoptosis and cell cycle is analyzed by flow cytometry (Zhang et al. 2002; Andreassen and Margolis 1994).

Inhibition activity of telomerase is based on the dose and time of cisplatin. BEL-7404 human hepatoma cells inhibited by 12 different concentrations of cisplatin (concentration range: 0.8–50 μm) in 72 h. During the treatment with cisplatin, it causes no change in the gene transcription as well as expression of hTR, hTERT, or TP1 mRNA in BEL-7404 human hepatoma cells. Cisplatin's treatment also arrests the cell cycle at the G1/M phase in BEL-7404 human hepatoma cells (Zhang et al. 2000).

10.6.9 Cisplatin and Lung Cancer

Lung cancer is also an excellent example in which cisplatin is a key antitumor chemotherapeutic drug used. Currently, platinum-based treatment plays a significant role in small cell lung cancer (SCLCs). In non-small-cell lung cancer (NSCLCs), cisplatin chemotherapy is used in the second and third stages at the primary or first stage of NSCLCs followed by routine surgery only (Dasari and Bernard Tchounwou 2014).

10.7 Conclusion

Cancer remains the primary cause of death regardless of notable advancement in the understanding of its molecular mechanism and the development of various treatments options. It has been years since the discovery of telomerase and its association with human tumor cells. A large number of inhibitors and drugs have been discovered or designed to date. Telomerase-based drugs can affect in a novel way, which layout new options for cancer therapy. On the other hand, telomerase inhibitors directly limit or stop the growth of human tumors and may act in a symbiotic manner with subsist therapeutic methods and amplify their effectiveness. Telomerase is the diagnostic and therapeutic biomarker as they are present in almost all cancer cells and are absent in most somatic cells. To explore the functions of telomerase in tumors and foreshadow the possibilities, it is being considered a future therapeutic target. Therefore, the discovery and design of new validated drugs towards telomerase is the priority.

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