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*Review Article*

# A role of telomerase in cancer progression and therapeutic program for anti-telomerase approach and future objectives

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## ABSTRACT

Typically, the length of the telomeres is shortened at each time of cell division, which can induce chromosomal instability. In many tumor cells, telomere lengths are maintained by an enzyme called telomerase, which is a mechanism for starting and surviving tumors. The human telomerase reverse transcriptase (*hTERT)* as the main subunit of telomerase enzymes in cancer cells is increased by various genetic and epigenetic mechanisms such as *hTERT* structural variants, *hTERT* promoter mutations and epigenetic modifications through *hTERT* promoter methylation. The *hTERT* upregulation in cancer cell propose the achievement that requires research into the mechanism of *hTERT* action in tumor cells.

***Keywords*:** Anti-aging therapy, *hTERT*, telomere, telomerase

## INTRODUCTION

Recent studies report that telomeres in humans are made of double-stranded DNA repeated with (TTAGGG)n sequence and with 10 kb long [1]. The polymerase, *an* enzyme inability to complete replicated the end of linear DNA, and the end replication problem regarding linear chromosomal DNA, so that the telomeres length are short at each time of cell division [2, 3]. In the early

stages of embryonic development, there is a specific DNA polymerase in human cells that can synthesize telomeric repeats and thus eliminate the problem of end replication and prolong cellular life. The essential function of telomeres with a shelterin complex to create a cap form in the end of the chromosomes to protect them [4, 5]. The shelterin complex include protein 1 (POT1) that protection role for telomeres and telomeric repeat binding factor: telomeric repeat-binding factor (TERF1 and TERF2). These proteins are interconnected with three others such as TERF1, Interacting Nuclear Factor 2 (TINF2), tripeptidylpeptidase 1 (TPP1), and repressor activator protein 1 (RAP1) [6, 7]. This complex has the ability to differ between telomerase from damaged DNAs site. In the absence of this protection by the shelterin complex, when the telomere is shortened to critical threshold, non-protective telomerase induce DNA damage response [8].

The components of the telomerase enzyme include, as follows: human telomerase reverse transcriptase (*hTERT*) as a catalytic subunit that plays a role in the synthesis of telomeric DNA from the RNA template and telomerase RNA componentas (*TERC*) an RNA template [9, 10]. The reverse transcriptase performance of this enzyme is by creates a 6-bases telomeric repeat using RNA hTR as a template. Moreover, recent studies indicate that the presence of active telomerase is apparently determined by the expression of *hTERT* and not by the level of hTR [11]. The TERT is the catalytic protein subunits that critical for telomerase enzyme and reverse transcriptase activity that encoded by the *hTERT* gene in humans [12, 13]. The *hTERT* is a 40 kb gene consisting of 15 introns and 16 exons [14]. It is located on the short arm of human chromosome 5 (5p15.33) approximately [14]. Only such as stem cells and progenitor cells that require high replicative, have a higher telomerase activity [15]. The *hTERT* has the ability to increase the proliferation of stem cells through the non-canonical pathway [15] .The RNA subunit are sufficient to increase the telomerase length and thus contribute to the formation of telomere nucleus [16]. At a certain amount of end-DNA digestion, damage-repair system recognizes the unprotected DNA double stranded as DNA breaks and activates the p53 or the p16INK4a signaling pathway to initiate a senescence or apoptosis program., which abound in all cells [17].

In overall, the effect of telomerase on the telomere length is regulated by the protein-counting mechanism, which is prevented access of telomerase more intensely by through the block the longer telomeres length contains higher levels of shelterin [18]. In

this study, role of telomerase in cancer progression and therapeutic program for anti-telomerase approach and future objectives was reviewed.

## The association between hTERT and tumor cells

The telomerase enzyme often acts in germline, hematopoietic, stem and renewing cells and does not activation in most somatic cells [19] which is due to its inactivity in somatic cells mainly due to tight *hTERT* regulation [20]. According to recent studies the *hTERT* is significantly expressed in human cancers and also telomerase upregulation is a critical feature in human cancers. Based on the activity of *hTERT* in a specific category of cells, telomerase activity might be a useful marker for diagnosis and monitoring (detect stage of cancer and outcome of disease) in different cancers including prostate, bladder, thyroid, breast, colon, gastric and lung [21-23]. Extensive studies have been conducted to found the mechanism of *hTERT* activity, but they mainly include mutations in the *hTERT* promoter [22]. Patients who carrying both mutations in *TERT* and *TERC* are related to an onset of disease is anticipated with increasing generations. Furthermore, the severity and the pathological condition of the disease correlates with the abundance of short telomeres [24]. Several mechanisms, including Myc and Wnt [25-27] which act as transcriptional regulators of telomerase, those oncogenes that cause telomerase transcription changes and activate telomerase, thereby maintaining the telomeric length and preventing cellular aging and apoptosis.

Different genetic mechanisms including *hTERT* promoter mutation, amplifications and *hTERT* structural variants plays a key role in the progression of tumor [28, 29]. In human tumors with telomerase activity, *hTERT* is one of the main targets for cancer cell proliferation during tumorigenesis [11]. The *hTERT* has a GC boxes that is very necessary for its promoter activity [21]. Two E-boxes (5-CACGTG-3), located at positions −165 and +44 of the nucleotide sequence of *hTERT* relative to the transcription start site (TSS). In addition, a single TSS is located in the core of the *hTERT* promoter bind to the transcription factor (TFII-I) that multifunctional transcription. Multiple transcription factors, as well as the telomere chromatin environment regulator of the transcription of the *hTERT* promoter. Several transcription factors binding that can act upregulate transcription including: c-MYC, SP1, E-twenty-six (ETS) family members, NF-kB, AP-2 and HIF-1 [21, 30]. A large study of 31 different types of cancer suggests

that 3 % of the *hTERT* expression indicated *hTERT* amplifications [31, 32]. Regarding the activation or repression of *hTERT* transcription by the *hTERT* promoter, the effective represses transcription factors include: TP53; represses transcription in an SP1-dependent manner), MAD (transcription factor involved in a network controlling cell cycle progression), WT1, MZF-2, SIP1 and menin have been shown to down-regulate *hTERT* transcription [33]. Recent studies reveal two common recurrence mutations with core promoter region of the *hTERT*, indicating a common mechanism for telomerase activity in cancer cells [34]. These mutations are usually, which occur at −124 bp and −146 bp upstream from the ATG start site, are C>T transitions (at positions 1,295,228 (C228T) and 1,295,250 (C250T) on chromosome 5). The recurrent *hTERT* promoter mutations occur in approximately 70 % of melanomas, 80–90 % of glioblastomas, 60 % of hepatocellular carcinomas, 60 % of bladder cancers, 70 % of basal cell carcinomas, 50 % of cutaneous squamous cell carcinomas, up to 30 % of thyroid cancers and approximately 72 % of oligodendrogliomas [34-37].

Genomic rearrangement is one of the effective mechanism in increasing the level of expression *hTERT* in the tumor that affects the *hTERT* gene locus (5p15.33). This rearrangement significantly increases activity of enhancers in proximity to the *hTERT* gene, and moreover the interaction between the promoter and the new induces enhancer drives *hTERT* expression. The new knowledge proposes to do the cloning of *hTERT* promoter and identification of various transcription factor-binding motifs, involved in *hTERT* expression and telomerase regulation by *TERTp*Mut [14, 38-42]. Also, bladder, thyroid, cutaneous melanoma, basal cell and squamous carcinoma and oligodendrogliomas are examples of cancers which *TERTp*Mut are widespread through different between various form of the disease, suggesting their role as an early tumorigenic event [43]. Additionally, not all *TERTp*Mut tumors display telomerase activation and some premalignant lesions also displayed these genetic alterations at the *hTERT* promoter region [44]. Important information came recently from a new study demonstrating that *TERT*pMut are necessary but not sufficient to maintain telomere length nor telomerase upregulation [21]. Moreover, *TERT*pMut usually occur in cancers with low rate of self-renewal such as brain tumors, liver, melanocytes and even low-grade bladder cancers suggesting a role in triggering telomerase activation. In adult gliomas, *TERT*pMut were found in 70 % to 80 % of glioblastomas, followed by

oligodendrogliomas (60 %) and oligoastrocytomas (35 %- 55 %). However, *TERT*pMut are rare events in ependymoma lesions [45]. Urological malignancies have a different prevalence of *TERT*pMut varying from rare or absent in prostate cancer and testicular germ cell tumors to high frequency between this cancers. In over 80 % of urothelial bladder cancer lesions, mutations are found to be one of the highest rate of genomic changes in bladder cancer [46]. In a recent study was performed on two non-coding mutations in the *hTERT* promoter region of the melanoma [34,43,47]. These two mutations were located at -124 and -146 bp upstream from ATG (chr5:1,295,228 G>A and 1,295,250 G>A, C>T on opposite strand).

Clinically, tumors carrying *TERT*pMut frequently express higher levels of *hTERT* mRNA and telomerase activity compared with those having a wild type promoter. Highlighting the prognostic potential of *TERT*pMut and the potential use as a clinical biomarker. Wu and his colleagues [48] reported an important co-occurrence of *TERT*pMut and TP53/RB1 mutations and suggested that they might cooperatively contribute to the progression of bladder cancer. Thyroid cancer patients with *TERT*pMut are associated with clinically aggressive and recurrent disease, with lower disease-free and overall survival when combined with *BRAF* mutations. Moreover, *TERT*pMut are a moderately prevalent genetic event in non-small cell lung cancer (NSCLC) associated with patient age, gender and distant metastasis [49]. Furthermore, current studies highlight the prognostic properties of *TERT*pMut and their potential use as a clinical biomarker.

## Epigenetic mechanism of hTERT promoter methylation

CpG dinucleotide sequences which DNA methylation occurred in its CpG site are spread throughout the genome, but there are specific regions known as CpG islands where high frequency of CpG dinucleotides is observed.

One of the first genes whose methylation of its promoter sequence was directly related to gene expression was the *hTERT* gene [50]. The methylation of the specific regions of the *hTERT* promoter, and especially the upstream regions of this promoter, has a significant associated with gene activation. Another repressor of *TERT* expression is Wilms tumor (WT) that it has a very high sensitivity binding to DNA sequence. The CpG methylation higher occurs in the WT1 binding sites in cancer. One of the ubiquitous transcription factors that are involved in controlling the process of cell proliferation

and differentiation is c-Myc that encoded by proto-oncogene c-Myc. The c-Myc has an important role in provoke of telomerase activity, c-Myc binding is also methylation-sensitive and its binding is reduced when binding site is methylated, resulting in reduced *hTERT* expression [51].

**Telomerase therapeutics**

Due to the presence of telomerase and its expression in human cancers and cancer stem cells, along with its absent of activity in normal cells, it has been suggested that the use of telomerase could be therapeutic proposed. A simple strategy for cancer cell death and progressive of telomere shortening could be inhibition of telomerase activity. Recent studies proposed that use of small-molecule inhibitors, antisense oligonucleotides and immunotherapy have been improved to decrease telomerase activity [52]. Induces apoptosis cell in cancer cell with minimum side effect on normal cells are the main goal of anti-telomerase therapeutics [53,54]. One of the competitive inhibitor of telomerase activity is imetelstat that was improved for the intravenous treatment of various cancers. It consists of a 13-mer N3–P5 thiophosphoramidate oligonucleotide that is covalently attached to a palmitoyl (C16 lipid) moiety through a 5-thio-phosphate group. In addition to oligonucleotide imetelstat (GRN163L) as an appropriate inhibitor of telomerase. Bryan and his colleagues introduced a BIBR1532 as a new inhibitor for telomerase, that binds to the domain of TERT and perturbation on TERT–RNA binding.

One of the main objective of telomerase immunotherapy is an inhibition of telomerase activity in cancer cells that act with the degradation of telomerase by proteasomes results in the formation of protein fragments or peptides of telomerase that are expressed on the tumor cell surface as antigens by the human leukocyte antigen (HLA) class I pathway [55,56], and these telomerase antigenic epitopes can be targeted by cytotoxic T cells to destroy the tumor cells [56]. CD4+ and CD8+ cytotoxic T-lymphocyte responses can be activated by telomerase-specific epitopes that finally capable of attacking tumors [57]. The highest amount of anti-tumor effect occurs in the presence of cells expressing *hTERT* peptides that activates the production of *hTERT* specific. The use of *hTERT* vaccine and dendritic cell approach has been developed as two important strategies to improve effective telomerase-based immunotherapy. GV1001, Vx001 and GRNVAC1 as a three *hTERT* vaccines are develop for anti-telomerase based on immune therapy in cancer patients. Between *hTERT* vaccines only one

therapeutic vaccine went all the way to the clinic (GV1001), and only one telomerase antagonist (imetelstat, GRN163L) is in late preclinical studies. One of the ways to expand the *hTERT* vaccine and to enter preclinical is to disrupt the active site of telomerase in cancer cells.

Adoptive cell therapy with the use of high-avidity T lymphocytes reactive against telomerase, has successfully been used in adenocarcinoma mouse prostate mice, which develop androgen-independent prostate cancer [58]. The study on the GV1001 vaccine is one of several studies on *hTERT* immunotherapy as an anticancer method in various cancers such as melanoma, glioblastoma, hepatocellular, leukemia, prostate, pancreatic, and non-small-cell lung cancer in different phases includeing 18 phases I/I-II studies, four phases II studies and one phase III trial with pancreatic cancer patients. Other methods have been proposed for future purposes, according to recent studies that includeing creating interactions between CD8+ and CD4+ T cells by immunogenicity of MHC class I and class II *hTERT* peptides to enhance pool of persisting memory CD8+ T cells; immunization with low affinity (mutant) MHC I *hTERT* peptide, limitation of the expansion of the immune-tolerance was achieved.

One of the main goal in gene therapy for cancer cells is promoter of telomerase that can selectively kill cancer cells and leave normal cells without harmed by expressing high concentrations of a therapeutic protein in cancer cells. Recent studies show the cancer cells using the mechanism of selectively replicate [59].

**Telomerase and one of the stimulation factor for anti-aging therapy**

A molecule called cycloastragenol commercially available as TA-65 that derived from a stragalus membrane root causes the telomerase to be activated transiently in the T lymphocytes and also can increase the proliferation and length of the telomere in a retardation of telomere shortening. ERK-pathway is one of the routes that increases the expression of telomerase and also cycloastragenol (TA-65) activates this pathway and subsequent increasing the expression of telomerase.

**Telomerase and drug resistance in cancer**

Lack of drug sensitivity of cancer cells and their ability to acquire resistance to anti-cancer drugs is one of the challenging problem in modern oncology. Recent data indicated that an activated DNA repair such as mismatch repair activation after the damage provoked by a drug in cancer cells

are another resistance mechanisms in those cells [60]. Various form of cancer type response drug resistance according to the category of a drug used in therapy. Also recent studies indicated that translocation of *hTERT* subunit to mitochondria is one of the drug sensitivity reason. In addition, previous studies have shown that mitochondrial enrichment with *hTERT* and as a result increased copy number of mtDNA can lead to suppress apoptosis and play a protective factor during treatment [61,62]. Two mechanisms that occur mainly after telomerase inhibition including DNA damage (drugs) and mismatch repair/genome stabilization. This indicates after therapy, the recurrence activity of telomerase in LoVo (colon cancer) cells that were accompanied by increasing telomere length and induction of telomerase subunit expression *hTERT* and hTR (human telomerase RNA or *TERC*).

## CONCLUSION

Studies indicate that telomerase enzyme activity is associated with cancer progression and telomere length increase, and can be used as an appropriate biomarker for cancer diagnosis. In fact, the recent findings suggest using interaction between telomerase and the shelterin complex and finally regulation of *hTERT* promoter, whether we can produce substrate to bind the active enzyme site and prevent to act. All of which indicate the importance of development of anti-cancer agent. Future studies need to increase our knowledge of the activity of *hTERT* in the early stages of carcinogenesis. Although recurrence mutation of the *hTERT* promoter in many cancers plays an important role in inducing the activity of telomerase. There are many advantages using telomerase as an anti-cancer target [63]. It is an essential component for most cancer cells and more widely expressed than any other tumor marker [64,65]. There are several challenges to use telomerase as therapeutic targets for cancer treatment including anti-proliferation of tumor suppressor that are induced in cells with a short telomerase length when drug is present. The side effects of proliferation of cells such as stem cells that require telomerase to survive. But one of the major problems is the time between drug management and clinical response. With the continuation of the therapeutic process, clinical success can occur, but it may cause intense toxicity in patients. Therefore, a major challenge to develop a telomerase inhibitor that rapidly kills telomerase-positive tumor cells while sparing normal telomerase-carrying cells.

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