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Telomere and Telomerase as Targets for Cancer Therapy

Xiaoping Tian · Bo Chen · Xiaochuan Liu

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Abstract Telomere maintenance and telomerase reactivation is essential for the transformation of most human cancer cells. Telomere shortening to the threshold length, mutations of the telomere-associated proteins, and/or telomerase RNA lead to telomeric dysfunction and therefore genomic instability. Telomerase up-regulation in 85% of human cancer cells has become a hallmark of cancers, hence a promising target for anticancer therapy. In this review, we discuss the mechanism of cancer due to telomere dysfunction and the resulting biological effects, the control of telomerase activity, and the new developments in cancer therapies targeting telomere and telomerase.

Keywords Telomere · Telomerase · Telomere dysfunction · Telomerase RNA · Cancer therapy

Telomeres, the DNA–protein complexes at the ends of eukaryotic chromosomes, are necessary for chromosomes stability through protecting the genome from degradation, end-to-end fusion, and inappropriate recombination [1]. Vertebrate telomere DNA consists of short, tandem repeated (TTAGGG)n sequences with G-overhang terminus. In mammalian cells, the double-stranded telomeric repeats are bound by a multiprotein complex known as *shelterin* or the *telosome*, which comprises tankyrase, telomeric repeat-binding factor 1 (TRF1), telomeric repeat-binding factor 2 (TRF2), TRF1-interacting protein 2 (TIN2), repressor-activator protein 1 (Rap1), protection of telomeres 1 (POT1), and TPP1(formerly named PTOP/PIP1/TINT1). Telomere terminates in a G-overhang bound by the Pot1/TPP1 heterodimer. Telomere folds back onto itself to form a double-stranded T-loop and a single-stranded D-loop [2]. Telomerase is a large ribonucleoprotein (RNP) reverse transcriptase, adding telomeric DNA repeats onto chromosome ends. Telomerase catalyzes telomeric elongation upon a single G-overhang strand. In telomerase-negative cells, telomeres could be maintained by alternative lengthening of

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X. Tian · B. Chen · X. Liu (✉)
Bioengineering Institute of Life Science College, Zhejiang Sci-Tech University,
Hangzhou 310018, China
e-mail: xcliu@zstu.edu.cn

telomeres (ALT) mechanism involving homologous recombination between telomeric repeats. The core enzyme of telomerase consists of catalytic reverse transcriptase protein (TERT) and telomerase RNA component (TER) [3]. Its activity is low or undetectable in most somatic cells, while up-regulated in about 85% of cancer cells and contributes to the immortality of cancer cells by maintaining the ends of chromosomes.

Telomere Dysfunction Associated with End Capping

Telomeres shorten with each cell division in telomerase-negative cells because of the "end-replication problem", resulting in cellular senescence due to insufficient telomeric DNA to form a stable telomeric complex. If the DNA damage pathways (such as p53, p21, p16INK4a, and pRb deficient) are suppressed, telomeres loss will lead to dysfunctional and critically short telomeres in a given chromosome, leading to genome fragmentation and cell crisis. Cells may stimulate the up-regulation of telomerase activity to extend telomeres, to escape this crisis, finally becoming immortalized [4].

Telomeric DNA terminates in a single G-overhang, which invades into the double strand forming the T-loop structure microscopically observed in mammalian cells [5]. The G-overhang varies in length among human cells, being significantly shorter in cancerous than normal endometrial [6]. It is suggested that G-overhang plays an important role in reserving telomere integrity and genome stability, supported by the fact that its length is much longer in aggressive endometrial cancers than non-aggressive cells. The results indicate that telomeres with longer G-overhang are more stabilized and cancer cells with such telomere have much more growth advantage than normal cells [7]. In addition, we thought that G-overhang is closely related to telomerase catalysis and telomeric end capping, worthy of further examination of terminal structure and associated proteins.

In mammalian cells, telomeric binding proteins include TRF1, TRF2, TIN2, Rap1, POT1, and TPP1 forming a multiprotein complex, termed as *shelterin* or *telosome* at the end [8]. TRF1 and TRF2 bind to the double-stranded DNA of telomeres, whereas POT1 binds to the single-stranded DNA portion. These proteins interact directly or indirectly with the proteins TIN2 and TPP1. A recent study suggested that TRF1 and TRF2 together play an integral role in T-loop and stabilizing [9]. TRF1 bends, loops, and pairs DNA to form the architecture of the duplex while TRF2 localizes to the D-loop junction to stabilize it in vitro.

TRF1 and TRF2 complexes also interact with each other to regulate the telomere length negatively [10, 11].TRF2 is the first telomere-associated protein involved in the maintenance of the correct DNA configuration of the telomeric G-overhang. Cells with TRF2 deletion exhibit telomere uncapping and end-to-end fusions, indicating its protective role in the telomere end-capping function [10]. Recently, it is reported that temperature-sensitive mutation in the Myb/SANT DNA-binding domain of TRF2 exhibits wide telomere damage, leading to the activation of the ataxia telangiectasia mutated (ATM) kinase and non-homologous end-joining (NHEJ) of chromosome ends [12]. It is noteworthy that TRF1 and TRF2 localized at the end cappings could be related with the terminal structure and/or cooperating with terminal proteins, such as POT1 only binding single-stranded DNA (ssDNA), though they could bind any region of double-strand telomeres.

POT1 is recruited to telomeres through its interaction with TPP1, and both proteins form a complex with TRF1. Biochemical analyses suggest that POT1 negatively regulates the telomerase activity by limiting access to its substrate. POT1 has two orthologs, Pot1a and Pot1b in the mouse genome. Evidence indicates that conditional deletion of the POT1a exposes the

chromosome terminal to the DNA damage response leading to p53-dependent senescence. In the p53 mutant background, Pot1a-deficient cells exhibit increased extelomere sister chromatid exchanges and telomere circles. This aberrant homologous recombination (HR) at telomeres results in chromosomal instability, promoting cellular transformation and tumor formation [13], which might be associated with telomeric diversity of given chromosomes.

Studies have shown that cells expressing TIN2 mutants lead to telomere uncapping and growth arrest, with telomere dysfunction and cell death in the p53-deficient background [14]. TPP1, putative mammalian homolog of telomere end-binding protein-beta, contains a predicted amino-terminal oligonucleotide/oligosaccharide binding fold. TPP1–POT1 association could increase POT1 affinity for telomeric ssDNA. Disruption of POT1–TPP1 interaction by dominant negative TPP1 expression or RNA interference (RNAi) triggered telomere-length alteration and DNA damage responses, demonstrating that TPP1 and POT1 function cooperatively to protect human telomeres by both positively and negatively regulating telomerase access to telomere DNA [15].

Expression of TRF1 and POT1 in trans results in extensive telomere elongation, while fusing TRF1 to POT1 abolishes this effect, inducing mild telomere shortening. Therefore, it is inferred that such a protein bridge between dsDNA and ssDNA may be responsible for the inhibition of telomerase access and promotion of telomere shortening [16]. Tankyrase 1 is a poly (ADP-ribose) polymerase poly (ADP-ribosyl)ates (PARsylates) TRF1, which causes the TRF1 to release from the telomere. It is reported that mutant tankyrase 1 with inactive ARC (ANK repeat cluster) IV, which is required for the TRF1 PARsylation, decreases the binding of POT1. POT1 is a downstream effecter of TRF1-mediated telomere length control. The mutant tankyrase1 loosens the closed structure of the telomeric heterochromatin and elongated the telomeric 3′-overhang as the wild-type tankyrase 1 did [17]. Thus poly (ADP-ribose) polymerase inhibitors may be used in tumor therapy.

Investigators have found that knockdown of TPP1 elicits a p53-dependent growth arrest and an ATM-dependent DNA damage response at telomeres. Thus, TPP1 depletion caused telomere dysfunction and induced chromosomal instability and tumorigenesis in the ATM-dependent DDR (DNA-damage response)-deficient background. The results indicate that loss of ATM combined with telomeres dysfunction promotes cellular transformation and tumor formation in vivo [18]. Cells with dysfunctional telomere show the hallmarks of ATM kinase signaling. In mammalian cells, damaged telomere can activate ataxia telangiectasia, ATM, and Rad3-related (ATR) which are inhibited by the TRF2 and POT1 of the *shelterin* complex, respectively. It is also discovered that ATM or ATR signaling is required for efficient non-homologous end-joining of dysfunctional telomeres [19].

DNA double-strand break (DSB) repair proteins are also required to cap the ends of mammalian chromosomes. DNA-dependent protein kinase (DNA-PK), a key component of the NHEJ pathway, was the first DSB repair protein shown to be required for capping the ends of mammalian chromosomes, and decreased levels of DNA-PKcs have been found in various human cancers [20]. DNA-PK is a nuclear serineythreoninekinase made up of two components, a 465-kDa catalytic subunit (DNA-PKcs) and the Ku heterodimer (Ku70, Ku80 are part of the most studied NHEJ pathway), and strains defective in Ku70 or Ku80 lose the majority of their terminal telomere repeats [21]. $TR^{+/-}$ cells with deletion of the gene encoding Ku70 proliferated more slowly than single mutant, frequent mitotic aberrations or rapid loss of G-overhangs. These findings suggest that Ku and telomerase function together to prevent homologous recombination of telomeres [22].

Either altering the telomere-associated proteins essential for the end-capping function or the telomere length critical for the maintenance of the proper structure through gradual or sudden loss of telomeric repeats can destroy the telomere function, as summarized in Fig. 1.

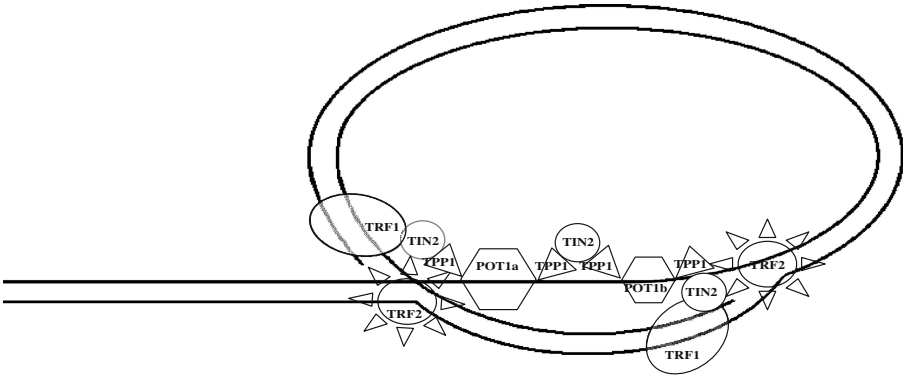

Fig. 1 TRF1 and TRF2 binds to the double-stranded DNA, while POT1 binds to the single-stranded DNA and has two orthologs. These three proteins interact directly or indirectly with TIN2 and TPP1. TRF1 bends, loops, and pairs DNA to form while the TRF2 localized at the two junctions to stabilize the loop structure

Telomere dysfunction results in the telomere end-to-end fusions and prolonged breakage/fusion/bridge (B/F/B) cycles (Fig. 2) and consecutive large-scale DNA amplification and terminal deletion. B/F/B cycles halts when the uncapped chromosome can acquire new telomere through recombination with the end of the other chromosomes [23–25]. This can cause on-going chromosome instability even after loss of a single telomere. Transformation of cultured human cells goes through three steps: (1) activation of proliferation by the proto-oncogene expression, (2) inactivation of the tumor suppressed genes, and (3) activation of telomerase activity by expression of the hTERT gene [26]. In such conditions, cells with fused chromosomes continue to divide, finally entering the crisis with short telomere. Rare cells with the re-activated telomerase continue to divide indefinitely and finally become immortalized. It is thought that telomeric DNA is transcriptionally silent because of its heterochromatic nature. Recently, it has been found that there is abundant G-rich RNA existing in mammalian cells. This abundance of the RNA is inversely correlated with telomere length and telomerase activity [27]. Therefore, it might be another factor contributing to cancer and aging.

Telomerase Regulation

The telomerase holoenzyme is composed primarily of two subunits, TER and catalytic subunit (TERT) and many associated proteins. TER is constitutively expressed in most somatic cells, while the catalytic subunit in higher expression is restricted to cancer cells. The holoenzyme adds the TTAGGG repeats onto the telomere by elongation and translocation. TERT catalyzes the elongation by adding the repeats complementary to the template region of the TER. The complex pauses and then translocates. The hTERT gene is mapped at the terminus of chromosome 5p [28]. It remains a question whether the telomerase is regulated by the telomere position effect that results in the reversible silence of genes near the telomere [29, 30]. Studies on both telomerase-positive and -negative cells have shown that the level of hTERT messenger RNA (mRNA) is in direct proportion to telomerase activity in some particular cell types [31–34]. The TERT gene expression is most likely regulated by transcriptional or RNA posttranscriptional modification [35]. It has been reported that c-Myc-activated telomerase through interacting with the TERT

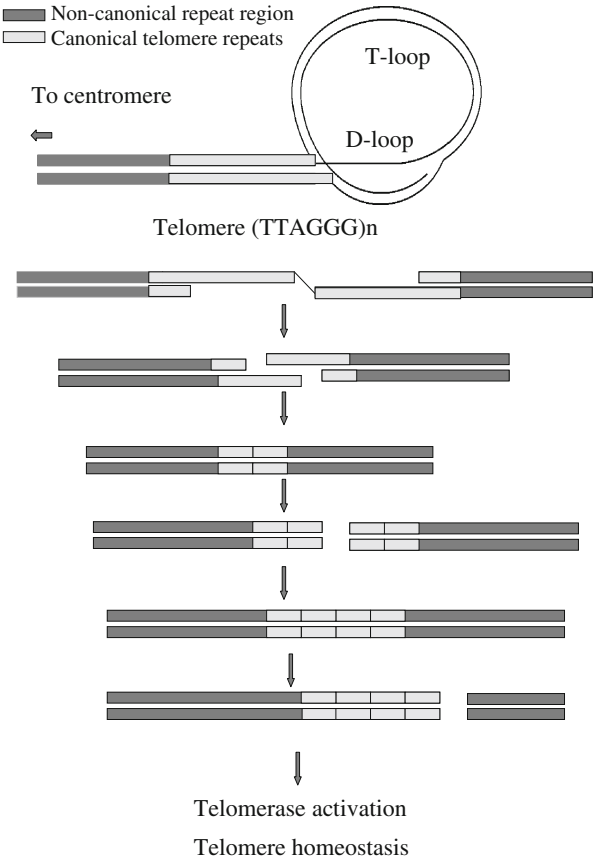

Fig. 2 Telomere becomes unprotected under a critical short length and recombines with each other, DNA damage response pathways are activated, and cells go replicative senescence (M1). In the deficiency of cell cycle checkpoints, cells with fused chromosomes continue to divide. Telomere continues to shorten leading to end fusions, M2 crisis. The fused chromosomes broken up at anaphase not exactly at the site of fusions. The sister chromatids undergo fusions after replication initiate the B/F/B cycles. The fused chromosomes broke up with DNA amplification and terminal deletions. The telomerase is reactivated and the telomere length is maintained in homeostasis (see [25])

gene promoter has promoted transformation by facilitating immortalization, thus becoming a hallmark of human cancer [25]. High levels of hTERT were found to express in undifferentiated embryonic human teratocarcinoma cells and its promoter was unmethylated in these cells. These studies indicate that histone deacetylation down-regulates the hTERT gene initially and DNA-methylated hTERT makes the hTERT gene silent [36]. It is supposed that the activation of the hTERT promoter may be caused by the feedback signal of telomere shortening.

Biological Effects of Telomerase Component Mutation

The telomerase RNA has a conserved secondary core structure among eukaryotes which comprises four conformational domains, the core (pseudoknot), CR4-CR5, box H/ACA,

and CR7 domains [37]. The proposed pseudoknots, adjacent to the template sequence, are created by the four short helices (P2a.1, P2a, P2b, and P3) [38]. In budding yeast *Kluyveromyces lactis*, some mutations of the pseudoknot region can reduce or ablate the telomerase activity. Other mutations in this region induce the incomplete copying of the telomerase RNA template. Hence, mutant telomeric DNA repeats are added to the terminal of the chromosome, leading to cell growth arrest and apoptosis [39]. Some nucleotides in the pseudoknot domain mediate the incompatibility between human TERT and mouse telomerase RNA [40].

In *Tetrahymena* and yeast, telomerase RNA template mutations result in mutant telomere to synthesize followed by cell growth arrest [41]. Moreover, some specific point mutations in the template domain even abolish the telomerase activity. Studies show that even a low threshold expression of mutant-template human telomerase RNA in prostate (LNCaP) and breast (MCF-7) cancer cell lines diminish the cell viability and increase apoptosis [42]. In human immortal cells, ectopic expression of the mutant hTR decreases the plating efficiency and growth rates, increasing the number of senescent cells. Recently, it is reported that cancer cells with mutant hTR are more sensitive to antitumor agents independent of their initial telomere length. Telomere maintenance mechanisms and overall telomere shortening are also not required [43].

The essential telomerase components include the TERT, the telomerase RNA component (TERC), and the TERC-binding protein dyskerin which binds the H/ACA box of human telomerase RNA and is a core telomerase subunit required for RNP biogenesis and enzyme function in vivo [44]. Studies have shown that mutations in these subunits resulted in dyskeratosis congenita (DC), which is characterized by such features as mucocutaneous abnormalities, bone marrow failure, and an increased predisposition to cancer [45, 46]. DC is proposed to be caused by the chromosome instability due to telomerase deficiency resulting from dyskeratosis congenita 1, TERC, and TERT mutations and is thought to be a disease of defective telomere maintenance [47]. It has been identified that ATPases pontin and reptin as telomerase components play an important role in telomerase assembly because their deletion can impair telomerase RNP accumulation [48]. This has resulted in an alternative method for cancer therapy.

Mutations of the telomerase RNA template resulted in correspondingly mutated telomeric DNA sequences to be added to telomeres in vivo. This may reduce the affinity of the sequence-specific binding proteins which are important for the capping function of telomere, thus leading to telomere end-to-end fusions and genome instability. The cell cycle checkpoint is activated with consequential cell growth arrest and senescence. In telomerase RNA mutant human cancers, however, its cellular response is different from DNA damage response: p53 is not required. Alternatively, with the inefficiency of p53 or Rb background, cells with fused telomere continue to divide, eventually entering into the mortality stage 1 (M1) with a short telomere. Several cells can bypass this crisis and continue to divide into the mortality stage 2 (M2) with the telomerase activity re-activated and finally becoming immortalized. Whether cancer cells reactivate the telomerase or up-regulated telomerase activity causes the cancer is still unknown. It is supposed that the telomere length in cancer cell is shorter than that of normal cells, so it requires potent telomerase activity to maintain the telomere length and maybe a stress reaction of the cell.

Cancer Therapy Targeting Telomere and Telomerase

Telomerase activity is up-regulated in about 85% of human cancer cells, while undetectable in most human somatic cells. Telomere maintenance is critical for constitutive proliferation

of malignant cells and is designated as a hallmark of cancer. Thus, telomere and telomerase are becoming potential targets for cancer therapy. Recently, multiple methods have been investigated to target telomerase and telomere for cancer therapy and these include gene therapy, immunotherapy, small-molecule, and signaling pathway inhibitors.

Gene Therapy

Antisense gene therapies aim to target the hTERT mRNA or telomerase RNA based on hammerhead ribozymes or RNAi having a selective impact on telomerase-positive cells [49, 50]. These include antisense oligonucleotides, peptide nucleic acids (PNAs) and chemically modified PNAs, such as 2′-O-methyl RNA and 2′-O-methoxyethyl RNA, phosphorothioate (PS), N3′-P5′ phosphoramidate (NP), and N3′-P5′ thio-phosphoramidate (NPS) DNA [51]. A lipid-modified N3′→P5′ thio-phosphoramidate oligonucleotide (GRN163L), which is complementary to the template region of the RNA subunit of telomerase, can inhibit the telomerase activity and cancer cell growth [52, 53]. siRNA directed against c-Myc activating telomerase through interacting with the TERT gene promoter may have a potential anticancer value. It is reported that shRNA of c-Myc in Colo 320 cells effectively reduces telomere length and inhibits telomerase activity [54]. Recent evidence indicates that telomerase reverse transcriptase (hTERT) promotes tumor cell survival independent of telomerase activity. DU145 cells exposed to 2′-O-methyl-RNA phosphorothioate oligonucleotide targeting a splicing site within hTERT pre-mRNA induce almost complete inhibition of telomerase activity, cell growth, and apoptotic cell death without any telomere shortening, while targeting the template region of hTERC failed to interfere with cell proliferation [55]. The disadvantage of this method is that it requires a time-lag before the telomerase positive cells are killed. Another approach is suicide gene therapy, whereby telomerase-targeted vectors carrying the enzyme encoding gene are transfected into cells. Toxins are released after the pro-drug is added and only the telomerase positive cells are killed [56–59]. A third is oncolytic virus therapy wherein the replication of the oncolytic virus is controlled by the hTERT promoter, and thus, the oncolytic virus replicates in and lysis the cancer cells selectively [60, 61]. The only concern is that these approaches will kill the normal stem cells and the cancer stem cells. A hypothesis considers that cancer stem cells have the ability to self-renew and generate diverse types of cancer cells at the same time [62, 63]. Studies have shown that cancer stem cells have shorter telomere lengths than that of normal stem cells. The telomerase inhibitors trigger cancer stem cells to apoptosis with critical short telomere before normal stem cells [64].

Telomerase (hTERT) Immunotherapy

The expression and activity of telomerase is undetectable in most somatic cells by birth; thus, hTERT-specfic lymphocyte precursors may escape the election pressure of the immune system during development. hTERT is nearly a universal tumor antigen for $CD8^+$; therefore, telomerase has become a promising target for immunotherapy [65, 66]. hTERT-specific CTL kills a variety of tumor cell types and HLA alleles via the endogenous pathway, which causes an immediate cell death by apoptosis prior to other telomerase inhibitors, many of which have a delayed cellular effect. It is necessary to develop an immunotherapy that activates both $CD8^+$ and $CD4^+$ lymphocytes (exdogenous and endogenous pathways complementary to each other) to produce a better cell-mediated immune response. It is proposed that DNA vaccination would create a potent antigen effect, due to hTERT polyepitope presentation on both MHC-I and MHC-II, and therefore

activation of $CD8^+$ and $CD4^+$ T cell, respectively. The disadvantage of the hTERT immunotherapy is that cells with ALT may resist the treatment [67, 68].

Two telomerase cancer vaccines have been developed: GRNVAC1, the trial isolated dendritic cells from patients, plus RNA encoding the telomerase protein component and then returned to the patients' body to activate the cytotoxic T cells to kill the telomerase-positive tumor cells [66, 69–71]. GV1001 vaccine, derived from the active site of hTERT, can be recognized by the immune system which then reacts to kill the telomerase-expressing cancer cells [72].

Small-Molecule Telomerase Inhibitors

Most researchers aim to seek natural agents or synthesize chemical compounds to inhibit or abolish the telomerase activity in cancer cells and destruct the telomere maintenance mechanism, leading the cancer cells to growth senescence and apoptosis. Most of the telomerase inhibitors are aimed directly at the core component of telomerase: the TERT and the RNA component TERC.

BIBR1532{2-[(E)-3-naphtalen-2-yl-but-2-enoylamino]-benzoic acid}, small non-nucleotidic synthetic compound, is a non-competitive inhibitor which is distinct from nucleosidic compounds or antisense oligonucleotides [73]. It is proposed that BIBR1532 does not block the template copying steps but impairs translocation of the enzyme DNA substrate complex or may promote the enzyme dissociation from the DNA substrate during catalyzing. BIBR1532 targets directly the telomerase core components hTR and hTERT and induces the cancer cells to growth arrest with telomere shortening and dysfunction [74]. TNQX (2, 3, 7-trichloro-5-nitroquinoxaline) is a mixed-type non-competitive inhibitor, which can suppress the telomerase activity both significantly and selectively [75]. MCF7 cell line treated long term with a non-cytotoxic concentration of the TNQX resulted in progressive telomere erosion followed by chromosome abnormalities and senescence. The mechanism of telomerase inhibition by the TNQX is inducing chromosomal end-to-end fusion, thus leading to incomplete DNA replication and cell arrest in late S phase [76].

Pyrimethamine which induces apoptosis and matrix metalloproteinases (MMP) and potent telomerase inhibition in the prostate cell line is considered to be a chemopreventative agent in cancer [77–79]. Lately, it has been found that the "T-oligo", an oligonucleotide which has the homology sequence to the terminal telomeric DNA, is a telomerase inhibitor and has shown cytotoxic effects in multiple cancers and destruction of the T-loop structure followed by the DNA damage response likely pathway [80].

G-quadruplex-Interacting Ligands

Telomere G-overhang could form G-quadruplex structures in vivo. Ligands that selectively bind to G-quadruplex, which stabilizes this structure, may interfere with telomere conformation and elongation [81]. The common features of these ligands include a large flat aromatic surface, presence of cationic charges, and the ability to adopt a terminal stacking mode. Derivatives of acridines, cationic porphyrins, triazines, anthraquinones, and perylenes, such as BRACO-19,TMPyP4, RHPS4, and telomestatin, can promote and stabilize the G-quadruplex structure, leading to disassociation of the telomere-associated proteins and cell apoptosis.

BRACO-19, 3, 6, 9-trisubstituted acridine compound, decreased hTERT expression drastically with the cellular senescence and complete cessation of growth in the human uterus carcinoma cell line UXF1138L. In vivo, BRACO-19 was highly active as a single

agent against early-stage tumors and with corresponding loss of nuclear hTERT protein expression and an increase in atypical mitoses indicative of telomere dysfunction. BRACO-19 is predicted to interact selectively with the human DNA quadruplex structure thus inhibiting the capping and catalytic functions of telomerase [82, 83].

Telomestatin, a natural product isolated from *Streptomyces anulatus* 3533-SV4, interacts preferentially with intramolecular in the absence of monovalent cations and also has a 70-fold selectivity for intramolecular G-quadruplex structures over duplex DNA. It stabilizes the intramolecular G-quadruplex structures and causes telomerase inhibition and shortening [84]. Telomestatin also provokes disassociation of POT1 and TRF2 from telomere causing telomere uncapping [85]. TMPyP4 tetra-(N-methyl-4-pyridyl) porphyrin facilitates the formation of intermolecular G-quadruplex structures, inducing the formation of anaphase bridges [84]. Another mechanism is that TMPyP4 down-regulates c-MYC which regulates the gene of hTERT, thus reducing the telomerase activity [86]. Pentacyclic acridinium methosulfate salt RHPS4, which can induce and stabilize the telomeric overhang to fold into a G-quadruplex structure, inhibits the catalytic and capping functions of telomerase [87]. Trisubstituted acridine derivatives are potent and selective telomerase inhibitors, such as those substituted on the acridine ring at the 2, 6, 9; 2, 7, 9; and 3, 6, 9 positions, which stabilize intermediate quadruplex structures that inhibit the elongation of telomeric DNA by telomerase in tumor cells [88]. Additionally, some compounds have been developed to bind the G-quadruplex more selectively and less cytotoxicly, such as carbocyanine dyes DODC and a bifuryl compound DB832 [89, 90].

Signaling Pathway Inhibitor

A constitutive activated melanoma-associated retinopathy (MAR) signaling pathway due to the over-expression of some oncogene is widely observed in human cancer cells. Several studies have shown that abnormal MAR kinase activity has an impact on the TERT gene transcription. Epidermal growth factor enables to activate the telomerase activity through the activation of the hTERT promoter via the Ras/MEK/EPK pathway [91]. Several proteins, Ets and activator protein-1 (AP-1) included, can repress this signaling pathway and have an impact on the TERT gene transcription and the telomerase activity [92]. Protein kinase C (PKC) and B (Akt) can phosphorylate hTERT enhancing the telomerase activity, thus protein kinase inhibitors can also inhibit telomerase activity. Bis-indolylmaleimide I and H-7, the PKC inhibitors, have been shown to inhibit telomerase activity in nasopharyngeal and cervical cancer cells. Tumor necrosis factor alpha (TNF-alpha) is capable of translocating TERT from the cytoplasm to the nucleus with NF-kappaB p65 and maybe another target for cancer therapy.

Other Inhibitors

Azidothymidine nucleoside analogs are a chain-terminating inhibitor of telomerase [93]. EGCG and MST312 which are epicatechin derivatives, non-nucleoside inhibitors, can inhibit the telomerase directly [94]. Helenalin [95], chrolactomycin, UCS1025A, and radicicol can affect the telomerase directly. Heat shock protein 90 (Hsp90) associates with multiple mutated, chimeric, and over-expressed signaling proteins including telomerase, and stabilizes their function. Additionally, hsp90 associates with telomerase complex and facilitates the assembly of the telomerase. Hsp90 inhibitors, novobiocin and radicicol, interact with hsp90 causing the inactivation, destabilization, and degradation of hsp90 client proteins. Thus, the inhibitors inhibit many signaling pathways that cancer cells depend on

for growth and survival. [96]. 9cUAB30, X receptor-selective retinoic acid, decreases DNA methyltransferase and telomerase expression [97].

Conclusion

Telomere-capping function requires (a) the high-order DNA–protein complex which requires sufficient telomere length, (b) the double-stranded or single-strand overhang binding proteins, and (c) telomerase maintaining the equilibrium of telomere length. Graduation or sudden loss of the telomere repeats or mutations of the telomere-associated proteins especially the sequence specific binding proteins may lead to the destruction of telomere structure. Mutations of the telomerase RNA core domains, especially the template region, cause the mutant telomeric repeats to be added to the telomere ends, reducing the affinity of telomere DNA-specific binding proteins. Cells with such mutations exhibit genome instability manifested as end-to-end fusions, NEHJ or HR. In such conditions, cells go to senescence or apoptosis in p53 intact background. With the deficiency of DNA checkpoints, cells with genome instability go through the B/F/B cycles (Fig. 1) and finally become immortalized with indefinite dividing ability.

The telomerase activity which is correlated with the hTERT expression level is reactivated or up-regulated in about 85% of human cancer cells. The TERT is regulated by transcriptional or posttranscriptional RNA modification or signaling pathways. The TERT can be down-regulated by histone deacetylation or promoter methylation while up-regulated by *c-myc* over-expression. Histone acetylation, promoter unmethylation, *c-myc* over-expression can be seen in most human cancer cells. Whether cancer cells reactivated the telomerase or up-regulated telomerase activity caused the cancer is still unknown. It is supposed that the telomere length in cancer cells is shorter than that of normal cells, so it needed potent telomerase activity to maintain the telomere length, and this might be a stress reaction of the cell. Thus, telomere and telomerase are two important factors in tumor formation. In cells, the change of one factor might lead to a change in another. So therapies targeting one factor may have some effects on another.

Cancer therapies targeting the telomerase are mainly directed at the RNA component or TERT subunits, while targeting the telomere is mainly directed at the telomere structural maintenance mechanism or the G-quadruplex structure. Knocking out the telomerase RNA component or TERT mRNA with the RNAi or hammerhead ribozymes can inhibit telomerase activity. Gene cancer therapies using gene delivery vector controlled by the hTERT promoter are more specific and selective to cancer cells. Many chemical compounds targeting the telomerase or telomere have been developed. Inhibitors purely target the telomerase require a time lag before the telomeres shorten to a critical length. The cells with ALT mechanism may resist the treatment. Telomere-targeting agents uncap the telomere and induce a p53-independent telomere-associated DNA damage response. The effect is fast and can induce senescence as well as apoptosis. Insights into the telomere and telomerase and their relationship with cancer provide further opportunities for cancer therapy.

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