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Review Article

Telomere Length and Cancer

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Abstract

Telomeres, the protective caps at the ends of human chromosomes, shorten with each round of cell division, representing a counter in the form of a biological clock. Beyond 50 to 60 divisions, the protective function of the telomeres will become unsustainable, and cells will usually experience senescence and death. Loss of cell function is manifested in the form of aging and the onset of diseases, including cancer. Cancer cells have found a way around this by overexpressing an enzyme, called telomerase, which counteracts the telomere shortening, thus allowing the neoplastic cells to keep replicating. This narrative review outlines why telomeres undergo shortening and how cancer cells exploit and take advantage of that phenomenon. The fact that malignant cells derail the biological telomere clock could be targeted for therapeutic benefit. The review also highlights the diverse telomere-based strategies explored in cancer treatment.

Keywords: Cancer, Shelterin, Telomeres, Telomere shortening.

طول التيلومير والسرطان

الخلاصة

التيلوميرات، وهي الأغطية الواقية في نهايات الكروموسومات البشرية، يُختصر طولها مع كل جولة من جولات انقسام الخلايا، وبهذا تمثل عدادًا على شكل ساعة بيولوجية. وبعد ٥٠-٦٠ انقسامًا، تصبح الوظيفة الوقائية للتيلوميرات غير مستدامة وعادةً ما تعاني الخلايا من الشيخوخة والموت. ويتجلى فقدان وظيفة الخلية في الشيخوخة وبداية الأمراض بما في ذلك السرطان. وقد وجدت الخلايا السرطانية طريقة للتغلب على هذا من خلال الإفراط في التعبير عن إنزيم يسمى التيلوميريز، والذي يعاكس مفعوله تقصير التيلومير وبالتالي يسمح للخلايا السرطانية بالاستمرار في التكاثر. توضح هذه المراجعة السردية سبب انكماش التيلوميرات وكيف تستغل الخلايا السرطانية هذه الظاهرة وتستفيد منها. يمكن استهداف حقيقة أن الخلايا الخبيثة تعرقل الساعة البيولوجية للتيلومير لتحقيق فائدة علاجية. كما تشير المراجعة إلى الأساليب المختلفة القائمة على التيلومير والتي تم البحث فيها على علاج للسرطان.

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INTRODUCTION

Eukaryotic cells are characterized by their linear chromosomes residing in the nucleus, but this design, as opposed to circular shapes, comes at a cost. Their ends will be recognized as DNA breaks requiring protection from repair machinery entrusted to identify and mend broken DNA [1]. The development of telomeres, which act like aglets at the ends of shoelaces, provided caps at the tips of each chromosome, enabling the necessary shielding. Telomeres are long, conserved structures with a lot of guanine (G). Special proteins attach them to DNA, halting most DNA damage signals and initiating pathways repair. In humans a telomere consists of six nucleotide repeat sequences in the form 5'-TTAGGG-3', where T is thymine, A is adenine, and G is guanine. The length of the double-stranded DNA repeat sequence in humans, $(TTAGGG)_n$, typically ranges from 10 kb to 15 kb (kb is a kilobase, which is 1000 base pairs), ending with a 50-400 base single-stranded G-rich overhang (Figure 1) [2-4]. The G-rich overhang (G-overhang) is thought to provide a platform for the binding of proteins that are important for the construction and protection of telomeres [2]. The telomerase enzyme repairs the ends by making more telomeric repeats [5]. It does this by attaching to the single-strand G-overhang. The single-stranded 3`overhang of the telomere loops back and invades the duplex, thus disrupting the double helix and base pairing (Figure 1) [6]. The structure that is formed because of this curling back of the DNA is called the T-loop (telomere loop), which is, together with its associated proteins, forming a protective cap at the terminus, preventing it from being perceived as a double-strand break (DSB) [7,8]. The double-strand and the single-strand DNA parts of the telomere are both embedded in a complex, known as shelterin, which is made up of six proteins [9]. Shelterin prevents exposed chromosome ends from activating a network of signalling cascades known as DNA damage response (DDR) [1,10]. Additionally, there is a telomeric repeat-containing RNA (TERRA) within

the sub-telomere region that is involved in regulating the capping and maintenance of telomeres [11,12].

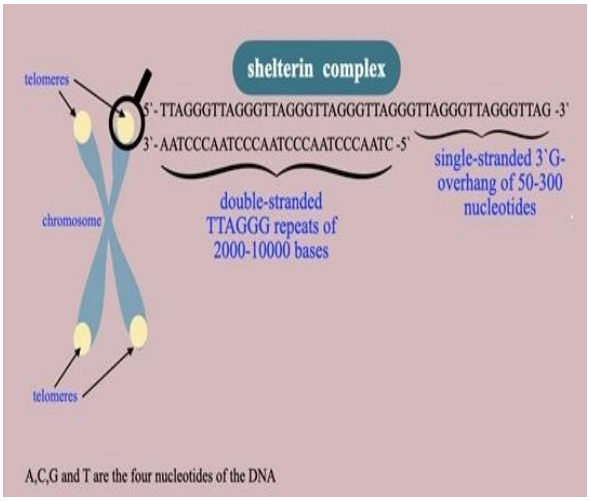

Figure 1: Expanded schematic view of the structure of human telomere.

METHODS

For this narrative review, literature searches were confined to the years June 1999–June 2024. Articles were examined employing the keywords given above and the search engines PubMed, Google Scholar, Web of Science, and ResearchGate. The first collection of publications was screened by the author, considering the citations of the manuscript and the impact factor of the journal. Studies that were considered to fall outside the scope of this basic narrative review were excluded. Publications before June 1999 were only considered if their content represented a significant and/or historic contribution to the topic.

A Brief Overview of DNA Replication

To fully appreciate the protective role of the telomere, it is useful to briefly review DNA replication. The copying of the DNA starts with the origin of replication, and there are multiple origins of replication on a human chromosome. The origin of replication is recognized by specialized cell machinery to form replication forks, which are areas of new DNA synthesis (Figures 2 and 3) [13]. Parental DNA strands separate at the ends of the forks to be used as templates for the synthesis of two new daughter strands. The two strands of the DNA double helix run in opposite directions, and the DNA polymerase replicating enzyme is needed to synthesize the new strand only in the 5’ to 3’ direction [14]. This is going to be problematic when it comes to copying the strand that does not get synthesized in the direction of the replication fork, which is known as the lagging strand. The DNA strand that will be copied in the direction of the moving replication fork is known as the leading strand. Experiments have shown that the leading strand is synthesized continuously in the direction of the overall DNA replication. It was found that the lagging strand is made up of Okazaki fragments, which are short pieces of DNA (about 100–200 bp) that are broken off. These fragments are made backwards to the direction of the replication fork [15,16]. The synthesis of the Okazaki fragments is primed by short pieces of RNA (3-10 nt) that are placed, *de novo*, by an enzyme called primase.

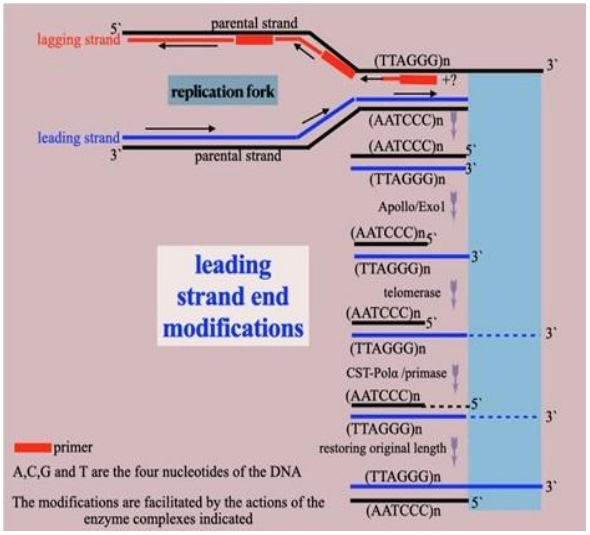

Figure 2: Telomere leading strand end modification illustrating the importance of telomerase enzyme (lacking in most human adult cells) to restore protective telomere length.

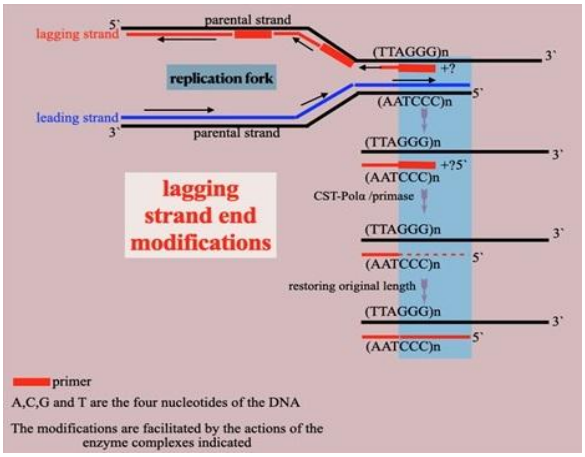

Figure 3: Telomere lagging strand end modification. The result could lead to telomere shortening depending on how much of the initial portion of the lagging strand was lost due to the position and subsequent removal of the last primer.

To form a continuous lagging strand of DNA, the RNA primers are first removed by the combined action of RNaseH, an enzyme that degrades the RNA, and a 5’ to 3’ exonuclease enzyme. The gaps are then filled by DNA polymerases, and the DNA pieces are then joined by DNA ligase that catalyzes the formation of a phosphodiester linkage between the 3-OH end of one nucleotide and the 5’-phosphate end of the other nucleotide. to yield an intact strand. In humans, leading strand synthesis is thought to be carried out by DNA polymerase ε (polε, epsilon), while lagging strand synthesis is started by a complex of primase and DNA polymerase α (polα, alpha), and the strand is extended by polymerase δ (polδ, delta) [17]. For over 50 years, scientists have realized that when DNA replication reaches the very tip of the linear chromosomes, there is a problem with copying the DNA [18,19]. Resolving this issue led to finding the connection between telomere length and replicative aging as well as cancer.

The "End Replication Problem"

The semiconservative replication of human chromosomes leads to a problem when it comes to the very end (termini) of each chromosome. This has traditionally been referred to as the "end replication problem" when it was first realized around 50 years ago [18,19]. At that time the problem was conceived as the inability to replicate a few nucleotides located at the termini of each chromosome after the RNA primer for the final Okazaki fragment had been removed (Figures 2 and 3). Upon the completion of the synthesis of the lagging strand, the removal of the final RNA primer should result in a potentially irreparable loss of a small portion of DNA and the formation of a short G-overhang of at least the same length as the primer (9-12 nt) if that primer was placed at the end of the daughter strand [14,20]. This nucleotide loss, from what is called the C-strand, is due to the inability of replication machinery to support the synthesis of Okazaki fragments using the G-overhang as a template [21]. *In vitro* work revealed the last Okazaki fragment is initiated by polα/primase well before (> 40 nucleotides before) the end of the duplex DNA, thus leaving the newly synthesized daughter 5' strand >40 nucleotides shorter than the original parental strand [21]. This cumulative DNA loss cannot be counteracted by telomerase enzyme and might become incompatible with genome integrity over successive rounds of replications. However, a resection was observed in which a segment of the "5' end" of the C-strand was removed before being refilled to essentially restore a longer G-overhang [22]. C-strand resection is usually performed by fill-in synthesis employing CST-polα/primase (Figures 2 and 3). The leading strand telomere faces a different problem every time a chromosome is replicated, as DNA synthesis on this strand generates a blunt end necessitating another solution [20]. A blunt end chromosome is not protected because it lacks the 3'-overhang, which is necessary for the T-loop formation, the docking of telomerase, and the structural integrity of telomeres. The solution here involves the resection of part of the 5' end of the leading strand in a manner not too dissimilar to the DSB repair and is carried out by Apollo/Exo1 enzymes [22-25]. This resection takes place about an hour after the replication, which is far behind the appearance of the G-overhangs on the lagging strand telomere [26]. Here, the resection of the C-strand would mean shortening of the G-strand of the leading end telomere by the length of the original 3`overhang and represents an "end replication problem" that could be resolved by the action of telomerase [27,28]. The effect of the C-strand resection and the absence of this G-strand elongation underlie the shortening of the telomere by 50-100 bp per cell division in human cells lacking telomerase function [26,29]. Unprotected telomeres and the loss of the G-rich overhangs result in end-to-end fusion of chromosomes, which can be catastrophic for genome stability, and for that reason, maintaining and shielding the termini is crucial to the cell [14]. The shelterin complex cannot protect telomeres that are too short. Loss of this protection can lead to the activation of the DNA damage response and either the death of the cell through apoptosis or the halting of its division through a process called senescence. These mechanisms will act as a safeguard against the development of cancer while maintaining adequate telomere length for tissue renewal throughout normal lifespan [30,31]. To counterbalance the ability to maintain a functioning genome against the need for adequate DNA damage response due to loss of telomere protection, cells employ an enzyme called telomerase. This enzyme is a reverse transcriptase that is a ribonucleoprotein (protein and RNA) in nature. Its RNA component is called TER (stands for telomerase RNA), and its protein component is a reverse transcriptase domain (TERT: telomerase reverse transcriptase). Telomerase uses its C-rich RNA template part to synthesize DNA and hence can only make G-rich DNA. Telomerase can be used by cells to add and extend the G-overhangs to telomeres, thus prolonging the viability of chromosomal tips before the cell experiences death or senescence. Telomerase requires at least 6 nucleotides of single-stranded G-overhang to function and can only add about 44 nucleotides [32,33]. In humans, cells only have the benefits of telomerase-mediated elongation if they rely on continuous proliferation, such as stem cells [34]. In most tissues, however, telomerase is downregulated and hardly expressed, and its absence results in the slow erosion of chromosomal termini with each replication cycle.

Telomere Shortening and Cancer

In the early development of a human embryo and some adult stem cells, the shelterin complex and telomerase collaborate to form a range of telomere lengths. The length of a telomere shows substantial differences among humans and even between individual telomeres [35]. A telomere length of around 1000 repeats (i.e., around 6000 bp) would allow enough cell divisions, even in telomerase-deficient cells, during the average lifespan of a human being. However, more recent studies showed that the terminal Okazaki fragment is situated about 70-100 nucleotides from the presumed end of the daughter lagging strand synthesis. This has substantial implications, pointing to as much as 1000 nucleotides lost in just around 20 cell divisions [36]. Differentiated somatic cells that lack telomerase count down telomere length with each cell division because of the telomere shortening problem. This countdown of telomere length continues until a critically short telomere is reached, signaling the limit of proliferative ability [37]. The telomere's primary function is to ensure the protection of the ends of chromosomes so that they are not mistaken as DNA breaks. Loss of the telomere's vital protective function causes the telomere to undergo age-dependent incremental attrition. Short and/or dysfunctional telomeres are recognized as DNA double-stranded breaks driving the cell to activate a special program called DNA damage response (DDR), which leads to replicative senescence or apoptosis (Figure 4). Should replicative senescence be overcome through the inactivation of

p53 and other cell cycle-controlling proteins, genomic instability will ensue, resulting in a phenomenon called “crisis” [38,39].

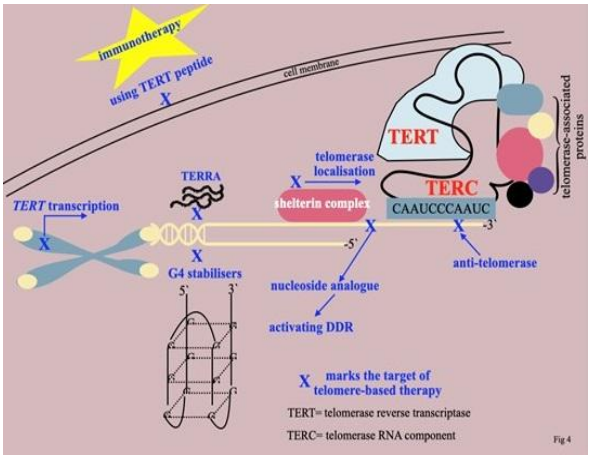

Figure 4: Targeting various components linked to telomere and its function in cancer therapy.

At this stage, a small population of cells acquires immortality through the activation of telomerase (or, in some other minor cases, through the alternative lengthening of telomeres (ALT) pathway) and proceeds in the path of carcinogenesis [38]. Most human cancers (around 90%) escape the telomere crisis through the activation of telomerase, with the remaining malignancies employing ALT [40]. Failure to undergo senescence following telomere shortening can result in a telomere crisis [41]. During a crisis, cells display a high level of genomic instability because of the presence of many dysfunctional telomeres and the lack of p53 and other cell cycle suppressor proteins. During this period of telomere crisis, cells undergo frequent apoptosis [42,43]. Cancer genomes are characterized by widespread genetic and epigenetic alterations of the telomere maintenance machinery. While longer telomeres are more advantageous for cell survival, cancer cells often, paradoxically, have shorter telomeres compared to those found in normal tissues. For example, it has been reported that the telomere length of prostate cancer cells is shorter compared to normal tissues [44,45]. Furthermore, in one study it was shown that 70% of cancer cell samples exhibited shorter telomeres compared to normal cell samples [46]. Aviv et al. proposed a theory based on two-stage clonal expansion through mutational hits to explain this apparent paradox [47]. The first hit at the stem cell level is largely telomere length-independent and generates a clone with replicative advantage and a second stage hit that is largely telomere length-dependent, transforming the expanding clones into cancer. The inhibition of telomerases, pharmacologically, in cancer cells leads to the gradual shortening of telomeres and the eventual senescence or apoptosis [48,49]. It was hypothesized that such an effect could emerge earlier in cancer cells with shorter telomeres, and the shorter telomere could be a useful predictive biomarker of telomerase inhibitors [50]. These observations suggest that cancer cells would be better off if telomere elongation by telomerase is abrogated. Once the cell machinery judges that extensive loss of the telomere protective caps has occurred, DDR is activated, and repair of the broken DNA is attempted. Mammalian cells can use two types of end-joining pathways to repair double-stranded DNA breaks, and these are as follows: A) classical non-homologous end joining (cNHEJ) and B) alternative non-homologous end joining (aNHEJ) [51]. The cNHEJ pathway results in accurate (or only small deletions) while the aNHEJ results in insertions and more extensive deletions. Telomerase activity is downregulated during human development mostly through the epigenetic silencing of the *TERT* gene encoding the reverse transcriptase subunit of the enzyme [52]. Other components of telomerase, including TERC, are expressed widely, making the expression of TERC alone sufficient to activate telomerase in many human cells [53]. As a result, most somatic cells, apart from certain stem cells, will undergo some degree of telomere shortening and the eventual induction of replicative senescence or apoptosis. The silencing of telomerase has the hallmark of being a tumor suppressor pathway, and for the cell to escape from this fate requires the reactivation of telomerase [54]. The expression of the *TERT* gene appears to bypass replicative senescence, implicating the continued cell division in telomere repair and length maintenance. Moreover, the overexpression of the shelterin subunit TRF2 can delay the onset of senescence [55]. The demonstration that telomerase is active in most human cancers but undetectable in normal tissues supports the role of telomerase in tumor suppression and the maintenance of most cancer types [56]. The telomeres of somatic cells gradually become shorter with each round of cell division, and after about 50-60 cell divisions, those cells possessing shorter telomeres will provoke replicative senescence [57,58]. A substantial proportion of human cancers (around 90%) reactivate telomerase through multiple genetic and epigenetic mechanisms, although the TERT promoter mutation is likely to be the most important mechanism [59-61]. Some types of cancer, like sarcomas and gliomas, don't have telomerase activity [46,62]. Instead, they show the alternative lengthening mechanism (ALT), which is a homologous recombination-based process.

Telomere-Based Targets in Cancer

The fact that telomerase is overexpressed in most cancers with minimal or non-existent expression in most somatic cells makes it a unique cancer biomarker and an attractive target for cancer therapy. For convenience and a better understanding of the therapies involved, the different approaches of targeting telomeres and their components can be classified into 1) direct targeting of telomerase using oligonucleotides and small molecules, 2) disrupting telomerase regulation and/or function, 3) employing immunotherapies that recognize the TERT part of telomerase as a tumor-associated antigen, 4) targeting *TERT* gene expression, and 5) inducing telomere dysfunction through the incorporation of nucleoside analogues at the newly extended telomeres (Figure 4).

Direct targeting of telomerase

The efficacy of inhibiting telomerase may be limited by the period of treatment required before obtaining sufficient telomere shortening to induce cell death. Telomere shortening requires several cell division cycles to become apparent, and treatment may have to be given for months to observe a therapeutically relevant tumor shrinkage. A direct telomerase inhibitor molecule called imetelstat has made it to clinical trials [63]. Imetelstat (GRN163L) is a 13-mer oligo (a short stretch of 13 nucleotides of RNA or DNA) complementary to the TERC template region that competitively inhibits telomerase activity [64,65]. The resulting suppression of telomerase activity was shown to reduce cancer cell viability *in vitro* and tumor growth in mice [66]. *In vitro* exposure of cancer cells to imetelstat causes the cells to undergo senescence and apoptosis after a period that is correlated with the initial telomere length [67]. Moreover, imetelstat also suppressed tumor growth in several mouse xenograft models in a predominantly telomere-length-dependent manner [66]. Imetelstat promotes gradual attrition of the telomeres, which results in the activation of DDR and cell death after a prolonged lag period. Patients with advanced non-small cell lung cancer showed no overall survival benefit using imetelstat in phase II clinical trials, although it yielded improved survival in those with the shortest telomeres [68]. As individuals with short telomeres are more prone to damage from radiation compared to those with long telomeres, imetelstat and other telomerase inhibitors that block telomere repair and elongation can enhance the killing of cancer cells through improved response to radiation [60]. A small molecule telomerase inhibitor called BIBR1532 has attracted interest when used in a preclinical setting as it selectively inhibits telomerase activity through binding to the active site of TERT [69]. It is a non-competitive inhibitor of telomerase mediating progressive telomere shortening and replicative senescence in cancer cells following extended treatments [70]. Although preclinical studies in breast cancer and prostate cancer cell lines have shown good results, the compound has not yet been included in clinical trials. High doses of BIBR1532 elicit cytotoxicity that is independent of telomere shortening, highlighting the potential value of developing strategies to trigger telomere "uncapping" (as opposed to telomere shortening) to obtain an anti-cancer response [71].

Indirect disruption of telomerase regulation and function

Guanine-rich DNA structures called G-quadruplexes (G-qs) sometimes form in the telomeres that can be resolved by helicases [72,73]. These structures, if not resolved, can disrupt telomere extension, and therefore stabilizing them can trigger DDR and cell death [63]. Preclinical studies have shown that stabilizers of G-quadruplexes can elicit anticancer effects, as in the case of telomestatin suppression of telomerase activity and tumor growth in leukemia xenograft models [74]. The G-q stabilizers shorten the 3' single-stranded ends of the telomeres, thereby indirectly inhibiting telomerase activity [60]. However, as there are around 300,000 such G-q sites in the human genome, the specificity of these stabilizers is yet to be thoroughly investigated [75]. Several other compounds, such as RHPS4 and BRACO19, have been shown to act as G-q stabilizers and can affect both the length and the structure of telomeres in a dose-dependent manner [65,67,76]. These compounds are thought to act by binding and sequestering the G-overhangs of telomeres and inducing telomere shortening through cell doublings [77]. It should be noted that the C-rich strand of the telomere is also able to form quadruplexes, known as i-motifs, and ligands for these structures might have preferential specificity for either the G or C quadruplexes [78]. Another approach to indirectly inhibiting the activity of telomerase is using nucleoside analogues as telomerase substrates. A modified nucleoside would not bind to components of the shelterin complex efficiently and would lead to telomerase dysfunction and rapid cell death. Acyclic nucleoside analogues such as aciclovir, ganciclovir, and penciclovir have been identified as inhibitors or antagonists of telomerase. Compounds such as 6-thio-2-deoxyguanosine (6-thio-dG, a telomerase substrate precursor) can rapidly induce telomerase dysfunction and cell death, thus avoiding prolonged telomerase inhibition and its consequent toxicity [79]. Due to the ability of 6-thio-dG to cross the blood/brain barrier, good results were obtained in the treatment of medulloblastoma in xenograft models [79]. Nucleoside analogues can act as "uncapping agents," impeding the binding of the shelterin complex to the telomere in telomerase-positive cells in a manner independent of the initial length of the telomere. The nucleoside analogue, 6-thio-dG, induces telomere dysfunction in telomerase-positive lung and colon cancers as well as BRAF-mutant melanoma cells [80,81]. Another compound that was found to be misincorporated into the telomeres to rapidly induce cell death in telomerase-positive cells is 5-fluoro-2`deoxyuridine triphosphate (5'-Fdu) [82]. This cancer drug was originally employed, and FDA-approved, for the treatment of colon and liver cancers. Compounds that inhibit telomerase catalytic activity, directly or indirectly, rely upon gradual telomere attrition with repeated rounds of DNA replication until critical telomere length triggers DDR, replicative senescence, and cell death [63]. However, the prolonged inhibition of telomerase can result in toxicity as well as the selection of ALT as an adaptation mechanism.

Targeting TERT gene expression

Researchers have already found that changes in the promoter of the "*TERT*" gene (which is part of the telomerase enzyme) are enough to keep the telomere length of human embryonic stem cells when they differentiate [83]. However, this has not been proved to be sufficient to activate telomerase in the context of differentiated cells, and such cells might require

concomitant mutations in the MAPK pathway [63]. The *TERT*-promoter mutations (TPMs) arise early in the tumor evolution [84]. The core *TERT* promoter contains GC boxes that are bound to the transcription factor SP1 (also known as specificity protein 1); thus, it is possible to regulate *TERT* transcription through the modulation of SP1 levels. Moreover, the *TERT* pre-mRNA is spliced into multiple isoforms, and only one of these isoforms encodes the catalytically active reverse transcriptase component of telomerase [85]. Knockdown of the main splicing factor, NOVA1, impaired telomerase activity and suppressed cancer cell growth *in vitro* and in xenografts [85].

Immunotherapy

Advancements in the field of immunotherapy have led to the finding that TERT-peptide is differentially expressed by cancer cells and can be recognized by HLA class I or class II molecules to trigger adaptive immune reactions [67,86]. Capitalizing on that discovery, several cancer vaccines that are based on TERT-peptide have been investigated and progressed to early-stage clinical trials. The cancer vaccine UV1 has shown an immune response in 86% of patients with prostate cancer [86,87]. One of the most advanced TERT vaccines is GV1001, which induces T cell responses in pancreatic cancer, NSCLC, and melanoma [88]. Another TERT-peptide vaccine is GX301, which consists of four different peptides and is considered more effective than a single peptide vaccine [89]. The use of immunotherapies combining TERT-peptide vaccines with immune checkpoint inhibitors has provided further support in targeting telomere shortening in cancer [63,67,90]. Assorting patients according to their *TERT* expression can further boost immunotherapy responses. Adoptive cell therapy has also been investigated as another aspect of immunotherapy to deal with telomere shortening in cancer. High avidity cytotoxic T cells that are telomerase–specific have been shown to suppress tumor growth and improve survival of mouse cancer models [91]. Targeting the *TERT* gene in dendritic cells (DCs) has also been considered and led to the arrival of the autologous GRNVAC1 vaccine, where patient-derived DCs are isolated and transfected *ex vivo* with mRNA encoding TERT [92]. Previous studies illustrated that GRNVAC1 is effective, safe, and well-tolerated in prostate cancer and acute myeloid leukaemia [65]. Plasmids containing the genes of the TERT peptide of interest can be delivered to antigen-presenting cells as in the case of the DNA-based vaccines phTERT and INVAC-1 that prolonged cancer survival times in human papillomavirus malignancy and melanoma tumor models [93,94]. Immunotherapies employing oncolytic viruses have exploited the high expression of telomerase in cancer. Telomelysin is an attenuated oncolytic adenovirus designed to selectively replicate in cancer cells through E1 gene expression driven by the human *TERT* promoter and induces lysis by overexpressing TERT [95]. Therapy using telomelysin resulted in cancer cell death and the sensitization of tumors to anti-PD-1 immunotherapy in mouse models of cancer [96]. However, limited antitumor response was observed in the phase 1 trial with this therapy, possibly pointing to the use of the human *TERT* promoter to control expression rather than the commonly mediated alterations of the *TERT* locus itself.

Other telomere-based targeting

The use of guanine-rich oligonucleotides (GROs) homologous to the 3’ single-strand overhang, also known as T-oligos, can be included under this heading. One such T-oligo is a specific 11-base sequence in the form 5’-GTTAGGGTTAG that induces DDR with minimal influence on the function of normal cells [60]. The *in vitro* application of T-oligos has been shown to reduce the growth of several cancers, possibly through the formation of G-q structures, the dissociation of the shelterin complex, and the aberrant upregulation of DDR [97]. The rapid degradation of T-oligos by nuclease enzymes is still a problem in their progression to clinical trials. The binding of TRF1 protein to telomeres has also been targeted using small molecules in lung carcinomas and glioblastoma through the induction of DDR [98]. Specific PI3K and AKT inhibitors were identified as being able to reduce TRF1, leading to an increase in telomere damage [99]. Indirect inhibition of telomerase can occur via the inhibition of tankyrase 1 (TNKS1, a protein that parsylates TRF1 during the S-phase of the cell cycle and displaces it from telomeres) [100-101]. Combination therapy using tankyrase 1 inhibitors and telomerase inhibitors may be an effective anticancer therapy. The shelterin protein TRF2 has also been implicated in several cancer-related pathways such as immune escape and angiogenesis [102,103]. One strategy under investigation is the use of peptides to disrupt TRF2 protein-protein interactions [104]. The most mutated gene in the shelterin complex is that encoding POT1, and mutations influencing its interaction with single-stranded DNA or TPP1 are associated with several cancers [105]. The bis-azo dye Congo red (CR) was shown to competitively inhibit POT1 binding to telomeric DNA *in vitro* [106]. Strategies employing microRNAs (miRNAs) to downregulate components of the shelterin complex to cause telomere dysfunction and thus cancer control have received attention in recent years. MiRNAs are a group of endogenous oligos that regulate gene expression at the post-translational level and effectively silence genes. Several miRNAs, such as miR-128, -138, -342, and -541, negatively regulate the expression of the *TERT* gene, thus acting as tumor suppressors, but their application is still in the preclinical stages [65]. The miR-155 was found to specifically target TRF1 and induce telomere fragility, a feature of TRF1 depletion in breast cancer [107]. Other microRNAs, such as miR-23a and miR-185, were found to target TRF2 and POT1, respectively, causing telomere dysfunction and replicative senescence [108,109]. TERRA plays an established role in telomere protection and genome integrity, with growing evidence attributing that as an anticancer

mechanism, and their transcription was shown to be affected by acridine and quindoline derivatives [110].

Conclusions

Previous work investigating the role of telomeres and their shortening with successive rounds of cell replications has provided a plethora of targets for the treatment of cancer. Capitalizing on that knowledge, research targeting telomere dysfunction in cancer will continue to gain ground to find suitable candidate molecules to progress to clinical trials and beyond. Currently, the amassed preliminary information in this field points to such products, once approved, being applied in conjunction with other cancer therapies to improve the overall benefit and patient's survival.

Conflict of interests

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Supplementary data can be shared with the corresponding author upon reasonable request.

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