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TELOMERE INHIBITION AND TELOMERE DISRUPTION AS PROCESSES FOR DRUG TARGETING

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■ **Abstract** The components and cofactors of the holoenzyme telomerase and its substrate telomeric DNA are attractive targets for anticancer agents that act by inhibiting the activity of telomerase. This review outlines recent advances in telomerase inhibition that have been achieved using antisense oligonucleotides and ribozymes that target the telomerase mRNA or its hTR RNA template. Although these are potent catalytic inhibitors of telomerase, they are challenging to implement in the clinic due to their delayed effectiveness. Drugs that directly bind to the telomeres, the complex structures that are associated at the telomeric ends, and stabilize secondary DNA structures such as G-quadruplexes are also potent inhibitors of telomerase. Special focus is given here to the telomeres, the biological machinery that works in tandem with telomerase to elongate telomeres, the causes of telomere disruption or dysfunction, and the consequences of disruption/dysfunction on the activity and design of anticancer agents.

INTRODUCTION

Human telomerase is a structurally complex ribonucleoprotein that is responsible for the maintenance of telomeric DNA at the ends of chromosomes. Telomerase acts to synthesize and add a simple six-base motif (of TTAGGG in the human case) to the ends of the chromosomes, resulting in stable telomere length that would otherwise be gradually eroded after each cell replication. Active telomerase has been detected in a majority of human cancer, embryonic, and germline cells but not in normal somatic cells, with the exception of some stem cells, such as those involved in tissue renewal.

The telomerase holoenzyme core consists of a catalytic subunit, the reverse transcriptase protein hTERT (1–3), and an RNA template subunit, hTR (4), which are essential for telomerase activity (5). Other proteins (6, 7) and kinases (8–11) are

important transient components of the holoenzyme and play a role in the activation, stabilization, and regulation of telomerase. Telomerase enzyme processivity is also dependent on the availability of the substrate telomeric DNA, dNTPs, and primer (12, 13).

This review explores the various approaches to and recent advances in the inhibition of telomerase, an enzyme whose activation and on-going activity are generally accepted to be paramount to the survival and proliferation of the majority of cancer cells. The processes and mechanisms of telomerase component assembly, activation, and activity have enabled the development of distinct targets and strategies for the inhibition of telomerase. These targets fall into three major categories that allow for the introduction of various agents to bring about the inhibition of telomerase activity. First, the processes of production and assembly of the elements necessary for telomerase activity yield potential targets, such as the genes (and therefore their messenger RNA) that express for the telomerase components, and the chaperone proteins and kinases that are required in the assembly process. Second, the active telomerase enzyme can be switched off by agents that target its components, hTERT and hTR, or other cofactors necessary for ongoing activity. Finally, agents can be introduced to bind to telomeric DNA, the substrate of telomerase, thus inhibiting telomerase activity by making it unavailable to the enzyme. Accordingly, this review deals with each of these important areas in turn; however, particular emphasis is given to the structure and role of the telomeres and the indicators of telomere disruption or dysfunction with a view to constructively examine the causal relationship among telomeric DNA length, cell senescence, and cell death.

THE TELOMERES

Structure and Function of Telomeres

Telomeres are specialized DNA protein structures that cap the ends of linear chromosomes. Mammalian telomeres consist of tandem repeats of the six nucleotides TTAGGG, which are repeated for 5–25 kilobase pairs in length 5′ to 3′ toward the chromosome end (14–17). Several recent studies have suggested that the structure of the ends of telomeres may be more complex than originally thought. Griffith and colleagues have found that telomeres do not end in a linear manner (18). Instead, the end of the telomere forms a loop structure with the 3′ G-rich strand (referred to as the T-loop), invading the duplex telomeric repeats and forming a displacement loop (D-loop) (Figure 1). Telomere-associated proteins may facilitate the formation and maintenance of both the T- and D-loops, suggesting the presence of a large DNA-protein structure at the end of each chromosome. In addition, most human telomeres appear to terminate in a single-stranded 3′ GT-rich overhang, which is thought to play an important role in telomere structure and function (19–21). These fundamental findings of the structure of telomeres have enormous implications for the design of compounds that can target and disrupt the telomere structure.

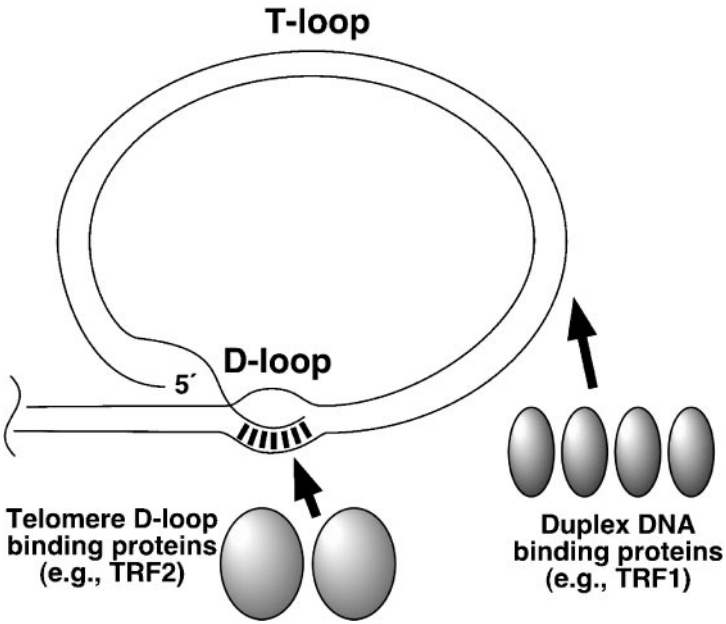


Figure 1 Cartoon showing the T-loop and D-loop and associated telomere binding proteins (18).

Telomeres are highly conserved in organisms ranging from unicellular eukaryotes to mammals, indicating a strong preservation of their protective mechanisms for preventing chromosomal ends from undergoing degradation and ligation with other chromosomes. Without telomeric caps, human chromosomes undergo end-to-end fusions and form dicentric and multicentric chromosomes (22–25). These abnormal chromosomes would break during mitosis, resulting in severe damage to the genome and the activation of DNA damage checkpoints. This leads to cell senescence or the initiation of apoptosis cell death pathways (26).

In addition to protecting chromosomes from end-to-end fusion, telomeres are also thought to protect against the loss of DNA at the end of each chromosome upon the completion of DNA replication. Dividing cells have been shown to undergo a progressive loss of 25–200 DNA base pairs following each cell division (22, 27, 28). This loss of telomeric DNA is largely due to the "end replication problem," which refers to the inability of the DNA replication machinery to copy the final few base pairs of the lagging strand during DNA synthesis (29, 30). Another possible cause for loss of telomeric sequence is by a 5′ to 3′ exonuclease activity that recesses the telomeric CA-rich strand (19, 31). Because the telomere consists of a repetitive DNA sequence, its loss is thought to be less important to the cell than the loss of critical gene encoding sequences that may be near the end of a

chromosome; therefore, telomeric sequences protect from the loss of more critical gene encoding sequences.

Crucial Characteristics Necessary for Senescence at the Telomeres

Telomerase has been thought of as an attractive anticancer drug target because of the compelling correlation between telomerase reactivation and cellular immortalization. The difficulty in designing therapeutic strategies with telomerase inhibitors is that to achieve telomere shortening, one would have to continuously treat the patient for multiple tumor cell population doublings. Most solid tumors have population doubling times of several days to several weeks, suggesting that anti-telomerase therapies could take months to produce any effect in a patient's cancer (32). These difficulties have slowed the development of telomerase inhibitors for the clinic. A key development in the approach to targeting telomeres is the discovery that alterations in telomere function, not the loss of telomere sequence, initiate cell crisis events such as replicative senescence (33). This understanding comes from the results of a study showing that inhibition of the function of telomere binding proteins leads to senescence in the absence of telomere sequence shortening (33), thereby implying that destabilization of the telomere may lead to the same effects that have been associated with critically short telomeres, namely, the induction of cell senescence and cell death.

The idea that telomere capping, not length, determines whether or not a telomere is functional has been proposed (34, 35). Telomere ends are capped by the binding of a number of telomeric proteins, including TRF-1 and TRF-2 (36). These proteins bind in a sequence-specific manner to telomeric DNA and protect chromosome ends from end-to-end fusion (25). The protection of the telomere ends is important to cell survival because loss of normal telomere capping leads to cell death by apoptosis (37). Response to the destabilization of telomeres appears to be mediated through an ataxia-telangiectasia mutated–dependent pathway, suggesting that unprotected telomeres can be recognized as DNA damage (37). Further evidence linking the telomere with DNA damage response pathways is the observation that components of DNA damage response pathways are localized at telomeres and are required for normal telomere maintenance. For example, the Ku protein involved in nonhomologous end-joining is localized to telomeres in budding yeast and human cells. Ku is involved in the localization of telomeres at nuclear pores, and loss of Ku function leads to end-to-end chromosome fusion (38, 39). Taken together, these results suggest that loss or uncapping of telomeric sequences can be sensed as a DNA break. It has been proposed that the DNA damage repair apparatus can allow for cell cycle checkpoint control so that cell cycle progression will not proceed until telomere replication is complete (40). The discovery of this monitoring system of telomere integrity within the cell leads to possible strategies to target telomere integrity for the treatment of cancer.

STRATEGIES FOR THE INHIBITION OF TELOMERASE

Control of the Production and Assembly of the Elements Necessary for Telomerase Activity

The assembly of the active telomerase enzyme complex in cells requires the catalytic hTERT protein and the hTR template RNA, components of the enzyme complex, as well as several accessory proteins, including Hsp90, p23, and TEP-1. Therefore, inhibition of any one of these proteins can lead to a decrease in telomerase activity, making these accessory factors potential molecular targets. Various strategies employing antisense oligonucleotides, ribozymes, or small molecules have been used to target telomerase by regulating the expression and activity of the proteins necessary for its assembly.

The Hsp90 and p23 proteins are molecular chaperones, which probably form a "foldosome" that facilitates and mediates the assembly of a biologically active telomerase complex (41). If the interaction of either Hsp90 or p23 with the catalytic subunit of telomerase is blocked, the assembly of active telomerase is blocked in vitro (41). Inhibition of Hsp90 function in cells with geldanamycin (an Hsp90 inhibitor) also blocks the assembly of active telomerase, suggesting that the targeting of the assembly of telomerase may be a very attractive way of inhibiting telomerase (41).

The hTERT messenger RNA has also been targeted with antisense oligonucleotides that are designed to hybridize with complementary sequences of hTERT mRNA. The recent clinical success of the first antisense drug provides the impetus for further development of these strategies for selective disruption of telomerase expression (42).

In addition to antisense targeting, hammerhead ribozymes have also been used to inhibit the expression of hTERT messenger RNA. These ribozymes are small catalytic RNA molecules that consist of a catalytic core flanked by antisense sequences that function in the recognition of the target sequence. These RNAs possess endoribonuclease activity that allows for the degradation of target transcripts. A hammerhead ribozyme has recently been used to cleave the hTERT mRNA in breast epithelial cells that inhibited telomerase activity and resulted in shortened telomeres, decreased net growth, and apoptosis (43).

Regulation of hTERT expression appears to be largely through transcriptional control (2, 3, 10, 44–46). Several transcription factors have been identified as having a role in the regulation of hTERT gene expression (47). Of note is the observation that c-*MYC* can induce telomerase activity by increasing the transcription of hTERT mRNA (48). There are c-*MYC/MAX* E-box binding sequences within the hTERT gene promoter, and several studies have addressed the importance of c-*MYC* in regulating the expression of telomerase in cancer cells (47, 49). Therefore, inhibition of c-*MYC* expression and interference with c-*MYC* regulation of gene expression are additional mechanisms for preventing telomerase activity (50, 50a). To explore this targeting strategy, we have recently identified compounds that can

down-regulate c-*MYC* expression, leading to repression of telomerase activity. These compounds recognize a specific G-quadruplex structure that forms in the nuclease hypersensitive element within the c-*MYC* promoter. The stabilization of this G-quadruplex leads to inhibition of c-*MYC* expression (50, 50a). Recently, a new DNA-binding small molecule, WP631 (Figure 2), has also been found to inhibit the transcription of c-*MYC* and *p53* genes that induce cell arrest at the G_2 checkpoint in the cell cycle and limited apoptosis in Jurkat T lymphocytes (51). These results are consistent with altered c-*MYC* expression by WP631 being linked to cell pathways leading to growth arrest in Jurkat T lymphocytes (51) that most likely involve inhibition of telomerase activity.

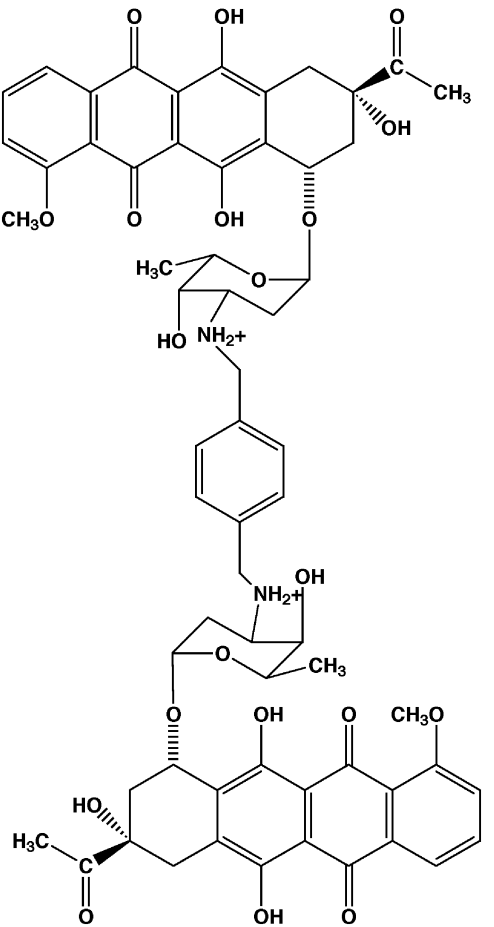


Figure 2 Structure of WP631.

Targeting of the Telomerase Components Necessary For and During Telomerase Activity

The intracellular introduction of synthetic molecules that bind to a specific component (or components) of the holoenzyme telomerase can serve to literally switch off the activity of telomerase once it is already in full-swing (as is the case in most cancer cells). To date, the agents employed to successfully down-regulate or inhibit telomerase activity by directly binding to one of its components have mainly targeted hTERT and hTR, the core subunits of telomerase. These inhibition agents are shown in Figures 3*A* and 3*B*.

Modified short DNA and RNA molecules with novel bond linkages between the bases have been designed with the aim to use the antisense approach by binding to the RNA template in the hTR subunit(s) to prevent or halt transcription and thereby act as competitive inhibitors of telomerase activity. Hence, the hTR RNA template is unavailable to hTERT for reverse transcription (52). The various types of sugar phosphodiester backbone modifications in these molecules are intended to confer certain desirable characteristics or properties, such as intracellular penetration, superior binding affinity, and therefore specificity, to the hTR RNA template and in order to enable intact delivery to their target.

In recent years, many researchers have extensively tested the efficacy of telomerase inhibition by peptide nucleic acids (PNAs) that contain *N*-(2-aminoethyl) glycine linkages between bases (52, 53), DNA-oligomers with phosphorothioate (PS) linkages (52), DNA-oligomers with phosphoramidite (PN) linkages (54, 55), RNA oligomers with methyl-substituted (56, 57) or methoxyethyl-substituted (58) ribose sugar rings (2-OMe RNA and 2-MOE RNA, respectively), locked nucleic acid RNA oligomers that have constraining ribose ring methylene bridges (54), various substituted RNA oligomers with PS linkages (56–58), and hybrid RNA–DNA molecules (termed as chimera molecules) consisting of a variety of combinations of the aforementioned oligomer types (54, 56–58). These agents are stable against intracellular degradation, such as nuclease digestion (54), and effect telomerase inhibition at the pico- to micromolar levels, depending on the cell line assayed (58, 59). Repeated transfection of cells with cationic lipids is required for delivery to cells and to maintain their effectiveness against telomerase activity (60). The impact of sustained treatment on telomere length, antiproliferation of cells, and even apoptosis (due to inhibition of telomerase activity) are all critically dependent on the initial telomere length and cell type (59). For example, in some cases treatment with agents such as substituted RNA oligomers prevented spontaneous immortalization of epithelial cells, even though cell apoptosis was not achieved (57).

The best of these agents are PNAs, which were shown to target specific regions in the hTR RNA template, with activities at pico- to nanomolar concentrations, an inhibition level 10 to 50 times more efficient than for PS-DNA oligomers (52). However, PNAs were found to have poor pharmacokinetic properties and higher toxicities relative to PS-containing oligomers (59). Also, PN-DNA oligomer derivatives containing complementary sequences to those at specific sites in the

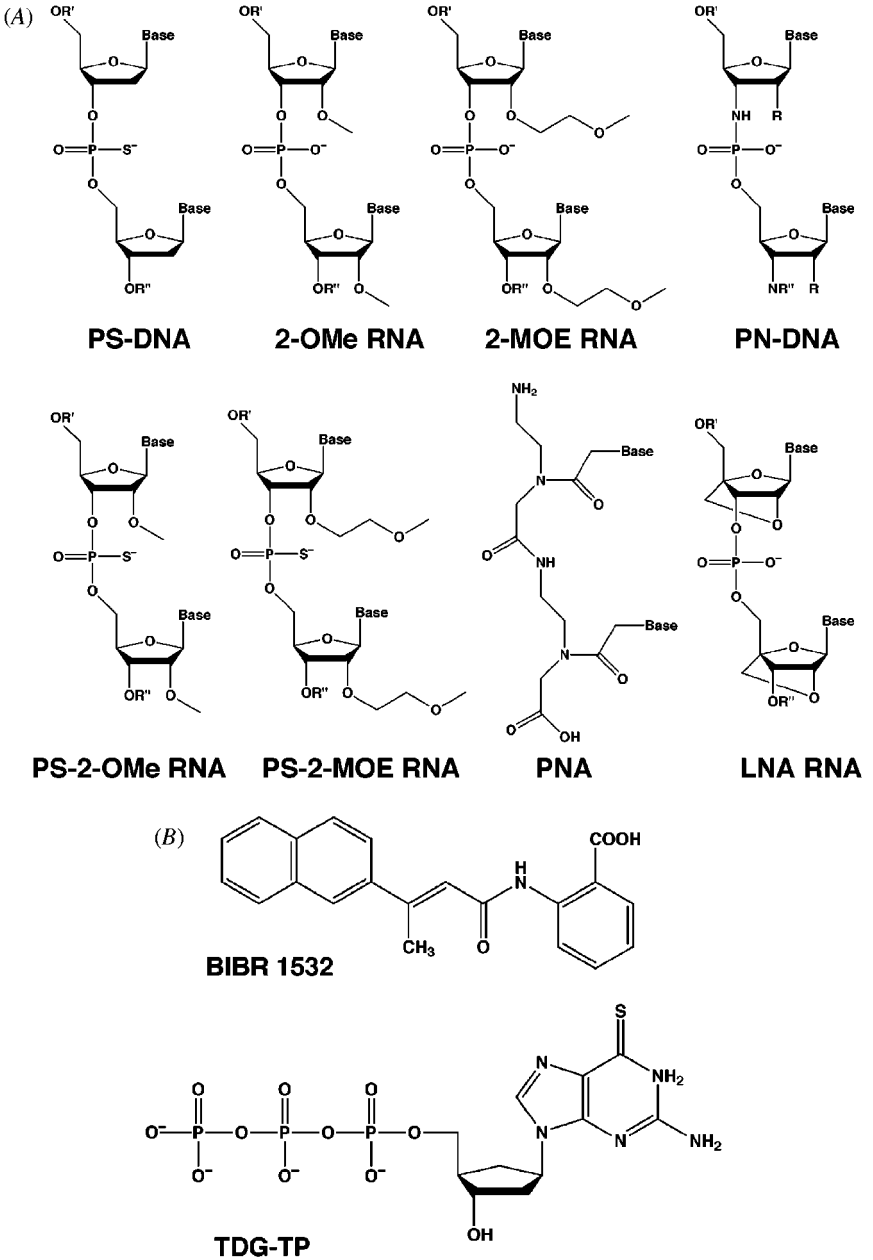


Figure 3 (*A*) The different types of antisense oligonucleotides that inhibit telomerase by targeting the hTR RNA template. (*B*) Two of the inhibitors of telomerase that target hTERT.

target hTR RNA template were efficient against telomerase activity at pico- to nanomolar concentrations (55). Interestingly, the PS-DNA oligomers have an enhanced nonspecific type of binding to proteins in general (59) and probably inhibit telomerase activity by interacting with the hTERT protein subunit of telomerase rather than with the hTR RNA template (59, 61). The 2-OMe and 2-MOE RNA oligomers and their DNA hybrids possess increased binding affinity at the target hTR RNA template and inhibit telomerase activity at the nanomolar level (56–58). The addition of PS linkages to these substituted RNA oligomers and hybrids confers better pharmacokinetic properties to these agents while still maintaining similar telomerase inhibition levels (56–58), giving them promising clinical properties (59).

Another promising approach employs a chimera molecule constituting an antisense DNA oligomer with an attached 2′,5′-oligoadenylate (2-5A) to induce massive apoptosis of ovarian cancer cells after sustained treatment over one to two weeks, but with no effect on normal ovarian cells (62). It is postulated that this chimera's antisense DNA oligomer enables direct binding to the hTR RNA template, while the 2′,5′-oligoadenylate recruits and activates an endoribonuclease (RNase L), which then cleaves the proximal hTR RNA template, thus inhibiting telomerase activity (62). Similarly, in several other studies the 2-5A antisense chimeras were used against prostate and bladder cancers and malignant gliomas in vitro and in vivo in nude mice with encouraging results (63–65).

Hammerhead ribozymes that cleave the hTR RNA template have been shown to inhibit telomerase activity in human (66) and endometrial carcinomas (67, 68) and in human melanoma cell extracts (69). Also, telomerase activity was downregulated in vitro in endometrial carcinoma and human melanoma cells (68, 69); however, no telomere shortening was observed in the human melanoma cells (69), and this approach to inhibition of telomerase activity has not been further investigated.

Telomerase activity can also be inhibited by the direct binding of small nonnucleosidic synthetic compounds to the hTERT reverse transcriptase component of telomerase. Schnapp and coworkers have recently reported the first mixed-type noncompetitive (70) catalytic telomerase inhibitor, (2-((E)-3-naphtalen-2-yl-but-2-enoylamino)-benzoic acid) (BIBR1532), which causes telomere shortening and senescence characteristics in various types of cancer cells in vitro and in vivo in mouse xenograft models at nanomolar concentrations (71).

Last, the hTERT reverse transcriptase inhibitors do not effect their activity by specifically and persistently binding to hTERT; rather, they act as competitors for the substrate deoxyribonucleotides used by reverse transcriptases, such as hTERT, to construct DNA chains (or more specifically for hTERT, to construct telomeric DNA extensions). Small nucleoside analogues can act as reverse transcriptase inhibitors, although only some of these compounds, such as 6-thio-2′-deoxyguanosine 5′-triphosphate (TDG-TP), are selective against the hTERT reverse transcriptase (72). TDG-TP is effective at low micromolar concentrations (72) and stops telomeric DNA extension after incorporation into the DNA (73).

Targeting of Telomeres

Inhibition of telomerase can be achieved by sequestration of the primer (the single-stranded telomeric end) required for the reverse transcriptase activity of this enzyme. This was first demonstrated by showing that K^+ inhibited telomere activity, presumably by facilitation of folding of the single-stranded telomeric DNA into a G-quadruplex structure (74). G-quadruplexes are composed of two or more G-tetrads (Figure 4*A*) assembled into either intermolecular (Figures 4*B,C*) or intramolecular (Figure 4*D*) structures. Human telomeric DNA can form an intramolecular G-quadruplex structure, characterized as a basket, having three G-tetrads, each stabilized by Hoogsteen base pairing (Figure 4*A*) (75). Based on the observations that K^+ facilitates and stabilizes G-quadruplex structures, small molecules that mimic the K^+ effect were also found to inhibit telomerase activity

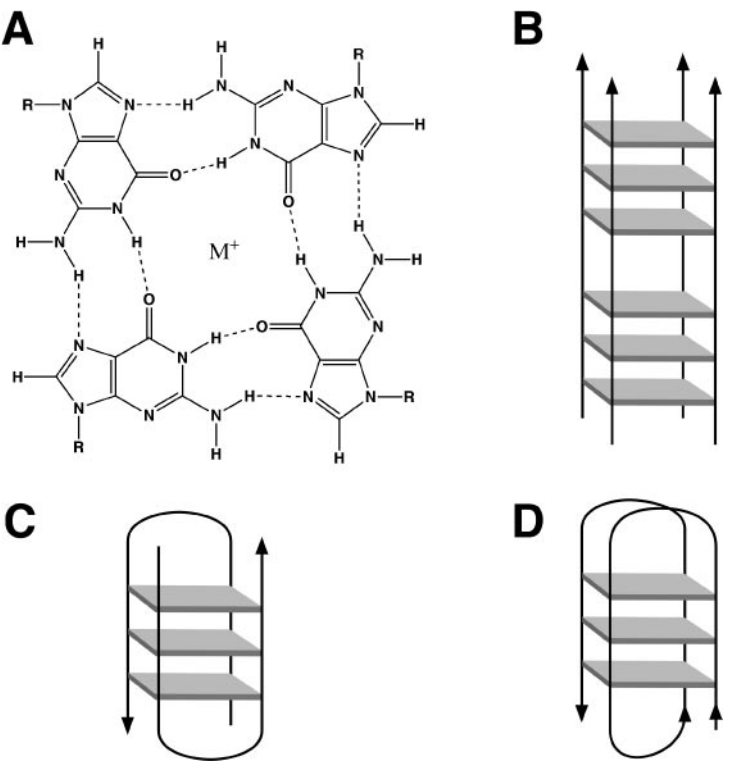


Figure 4 G-tetrad and G-quadruplexes. (*A*) Four guanine residues forming a planar structure G-tetrad through Hoogsteen hydrogen bonding. (*B*) A parallel G-quadruplex model. (*C*) An intermolecular antiparallel G-quadruplex model. (*D*) An intramolecular basket G-quadruplex model. Each parallelogram in (*B*), (*C*), and (*D*) represents a G-tetrad.

(76). The first proof of this principle was that telomerase inhibition did not take place until sufficient telomeric repeats were assembled by telomerase extension of the DNA primer to form a G-quadruplex structure (76). Consequently, the direct telomerase assay (77) (Figure 5), alongside the polymerase stop assay (78) (Figure 6), provides important cell-free signatures of G-quadruplex-interactive compounds. In addition to cell-free assays, in vitro and in vivo assays have also been used to characterize the effects of G-quadruplex-interactive compounds. In in vitro systems, inhibition of telomerase (79), telomere shortening (80), cell senescence, and delayed growth inhibition (80) have been demonstrated with G-quadruplex-interactive compounds. Unlike telomerase inhibitors, G-quadruplex-interactive compounds would be expected to affect cells that maintain telomeres by telomerase-dependent as well as telomerase-independent mechanisms because the latter involve recombination mechanisms quite possibly involving G-quadruplex structures (81). The formation of G-quadruplex structures in regions other than telomeres, for example, in the promoter region of c-myc, may also lead to effects on telomerase because c-myc controls hTERT. Indeed, the G-quadruplex-interactive compound TMPyP4, but not its isomer TMPyP2, which does not interact with G-quadruplex, is able to down-regulate c-myc and hTERT (50, 50a).

As described before, an altered telomere state may be a more important consequence than critical telomere shortening (33). Thus, disruption of telesomes by either depletion of proteins involved in telomere binding (e.g., telomerase) or sequestration of telomere ends by stabilization of G-quadruplex structures, or both, may lead to chromosome end-to-end fusion in presenescence cells. In fact, TMPyP4 and the fluoroquinophenoxazines have both been demonstrated to produce anaphase bridges, a hallmark of chromosome end fusions, in relatively short periods of time (78).

It has been frequently pointed out that G-quadruplex-interactive compounds lack the cancer cell selectivity imparted by the fairly selective occurrence of telomerase in cancer cells. However, uncapping and recapping of telomerase-dependent cells may provide some opportunities for selectivity of G-quadruplex-interactive compounds. In cells switching between these two states, this most likely occurs in S or G2/M phases in the cell cycle. In cancer cells, where it is necessary to add telomeric sequences via a telomerase-dependent or telomerase-independent mechanism, the single-stranded DNA template becomes exposed in the uncapped state, and if either telomerase is deficient or G-quadruplex-interactive compounds are present, which facilitate folding of the G-quadruplex (e.g., TMPyP4, telomestatin), irreversible uncapped telomeric ends and chromosome end-to-end fusion may result (78). In contrast, normal cells that do not need to elongate their ends may remain stable through multiple cell divisions (35).

The natural occurrence of G-quadruplex in human telomeric sequences remains unproven, although a recent report provides convincing evidence of their occurrence in *Stylonychia lemnae* telomeres (80). Nevertheless, the existence of

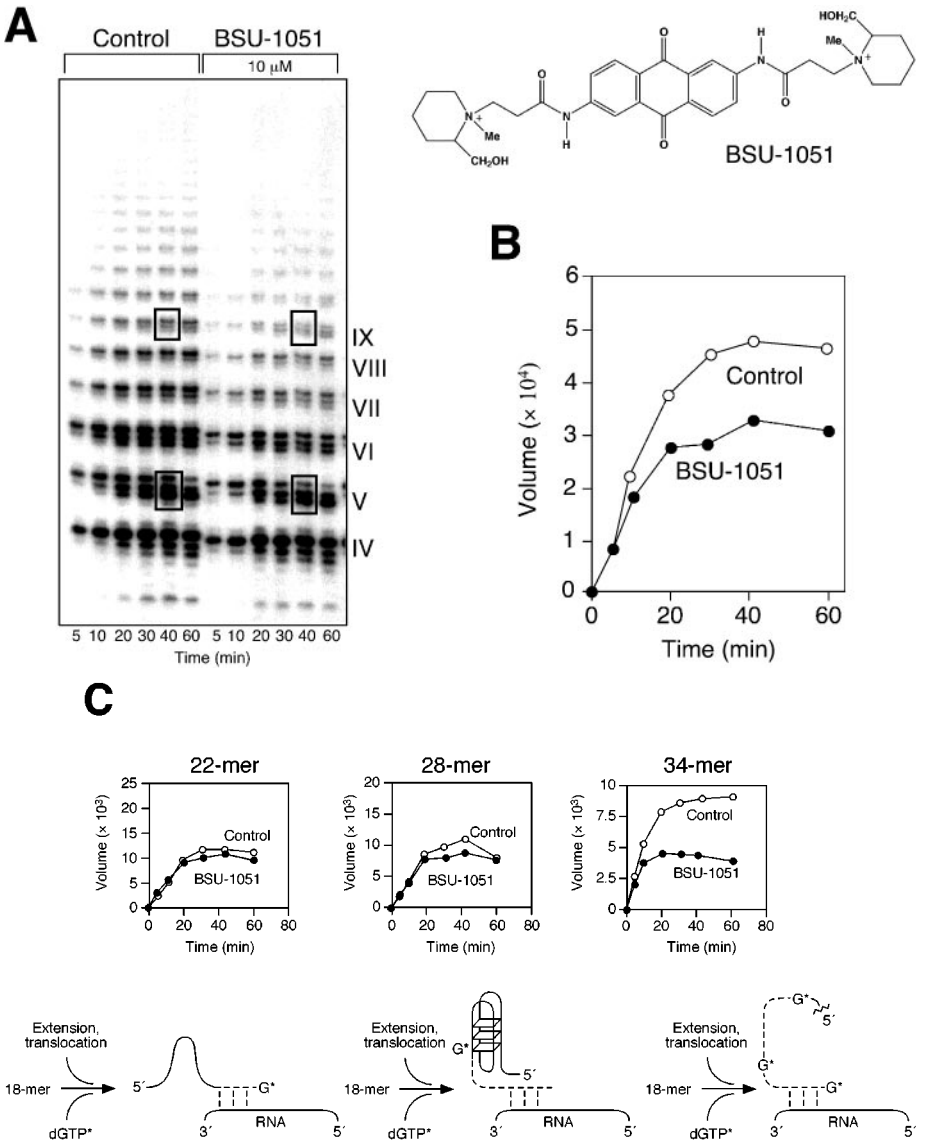


Figure 5 Effect of BSU-1051 on the time-course of telomerase activity using (*A*) the 18-mer telomeric primer d[TTAGGG]$_3$ (1 μM) without (*left-hand lanes*) or with (*right-hand lanes*) BSU-1051 added at 10 μM (76). The boxes identify the 40-min samples, which show altered multiple band patterns due to 3′-exonuclease activity. (*B*) Time-course of total amount of [α-^{32}P]-dGTP incorporated into the extension products of the d[TTAGGG]$_3$ primer in the presence and absence of BSU-1051. (*C*) Time-course incorporation of [α-^{32}P]-dGTP into the 22-mer, 28-mer, and 34-mer and comparison of patterns of sets of multimers for the 22-mer and 28-mer in the presence and absence of BSU-1051. The diagrams between the two sets of results show the proposed structures of the species formed at each step.

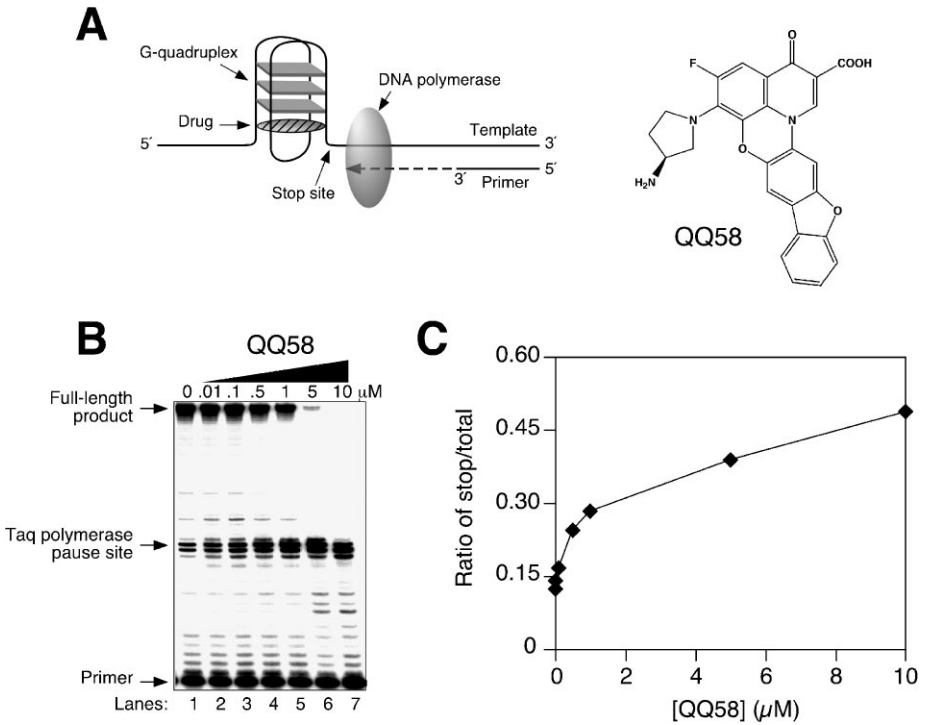


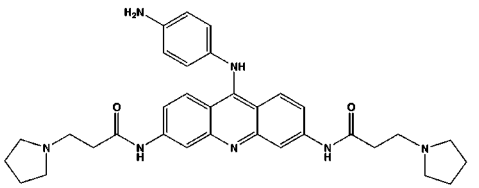
Figure 6 Inhibition of Taq polymerase with increasing concentrations of the fluoroquinophenoxazine QQ58. (*A*) Cartoon of the assay and structure of QQ58. (*B*) Autoradiogram of the sequencing gel showing enhanced DNA synthesis pausing at the G-quadruplex site with increasing concentrations of QQ58 (*lanes 1–7*). The free primer, the pause site, and the full-length product are indicated. (*C*) Graphical representation of the quantification of the sequencing gel shown in (*B*), showing the concentration of QQ58 to the ratio of intensity of the bands obtained for the pausing site/total intensity per lane.

helicases such as Sgs1 and Cdc13p in yeast and Werner's and Bloom's helicases in human cells, which resolve G-quadruplex structures, suggests that such structures may exist at least transiently (82–86). Whether or not these structures form naturally, it is likely that their formation and subsequent resolution need to be controlled so that free single-stranded telomeric primer can be available for telomerase reverse transcriptase. Therefore, if G-quadruplex-interactive compounds interfere with the dynamics of interconversion between G-quadruplex or single-stranded telomeric sequences, they will likely have effects on telomeric states of DNA. It was first demonstrated with a perylene (PIPER) that G-quadruplex-interactive molecules could facilitate the formation and also inhibit the Sgs1 helicase unwinding of G-quadruplex structures (87, 88). Subsequently, a number of other compounds, including the cationic porphyrins, telomestatin, and 9-anilino-proflavins, have been

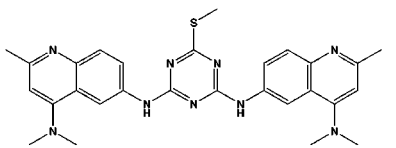
shown to facilitate or inhibit G-quadruplex conversion to single-stranded DNA (89–91).

The first G-quadruplex-interactive compounds were relatively nonpotent (IC_{50} in the micromolar range) (92) and nonselective for G-quadruplex versus other forms of DNA. More recently, more potent and selective molecules have been designed or discovered (78, 80, 90, 93–95). Of particular interest are 9-anilino proflavine, the triazines, the fluoroquinophenoxazines, telomestatin, and the pentacyclic acridines (Figure 7).

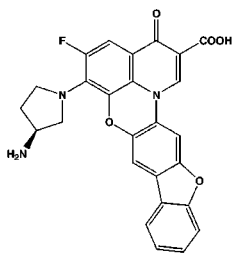
The 9-anilino proflavine derivative was designed to optimize the interaction with the intramolecular G-quadruplex from human telomere and minimize that with duplex DNA. These compounds have 60 to 100 nM potency in a modified TRAP assay and corresponding low cytotoxicity (93). The triazines have been demonstrated to produce telomere shortening, which is associated with delayed growth arrest and cell senescence (80). The fluoroquinophenoxazines are redesigned topoisomerase II poisons that now interact more specifically with G-quadruplex structures, and this activity is correlated with production of anaphase bridges (78), a property also shared by the cationic porphyrin TMPyP4 (96) and



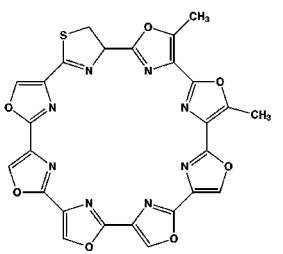
9-anilino proflavine



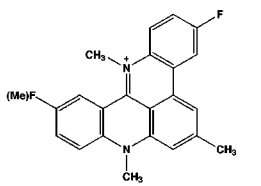
triazine (115405)



fluoroquinophenoxazine (QQ58)



telomestatin



pentacyclic acridine (RHPS4)

Figure 7 Chemical formulae of some of the inhibitors of telomerase that target its substrate (telomeres).

the triazines (80), both of which are also G-quadruplex-interactive compounds. Telomestatin, a natural product, is the most potent of the telomerase inhibitors (97) and also a G-quadruplex-interactive compound (90). This compound shows remarkable specificity for intramolecular versus intermolecular G-quadruplex structures. The pentacyclic acridine RHPS4, like the other G-quadruplex-interactive compounds reported above, produces cellular effects within a two- to three-week period at noncytotoxic concentrations. A number of reviews on drug targeting of G-quadruplex have appeared (60, 98–100).

In addition to the exciting developments described previously, leading to more potent and G-quadruplex-specific agents, underlying concepts for how G-quadruplex-interactive compounds may affect biological processes have been advanced. It is likely that, in most cases, the occurrence of G-quadruplex in genomic DNA is undesirable unless the facile conversion between duplex or single-stranded DNA and G-quadruplex DNA can be achieved (101). In the latter case, their involvement as switch signals in transcriptional control might be a useful and even primordial mechanism for regulation of gene expression. Therefore, agents such as the perylene and cationic porphyrins, which both facilitate the formation of G-quadruplex (91) and inhibit their resolution by helicases such as Sgs1 (87, 91), are likely to affect the natural equilibrium between duplex and single-stranded DNA and G-quadruplex structures, and thus have effects on biological processes mediated either by duplex or single-stranded DNA or by G-quadruplex DNA forms (101).

Last, Brad Chaires has developed an extremely useful method to determine the DNA structural selectivity for binding of ligands to different forms of DNA (102). Thus it is possible to determine if there is a correlation between binding affinities to G-quadruplex and biological activities for a range of analogues. However, for this to be predictive, the biologically relevant G-quadruplex must first be known.

CONCLUSIONS

Telomerase has alternately waxed and waned as a potential cancer-specific target. The first expectations following the discovery of telomerase were overly optimistic in accordance with the lack of appreciation of the processes that maintain telomere length. It is more than a decade since these halcyon days, but telomerase is now enjoying a revival as a more validated molecular target. The concept of altered telomere states that prompt chromosomal end-to-end fusion in presenescence cells, thus negating the need for critical telomere shortening, will affect the way we think about treating cancer cells with telomerase inhibitors and telomere-interactive compounds. For us to take full advantage of this new concept, we need more complete information on the uncapping and recapping of telomeres. Compounds such as the G-quadruplex-interactive drugs, which interfere with telomere structures and presumably produce altered telomere states, assume new

significance under this new insight. Moreover, compounds that lower c-myc and hTERT and interfere with telomere integrity assume even more significance. From the discovery of small organic compounds to larger synthetically modified oligonucleotides that target defined macromolecular structures, the field has moved to measuring effects on telomerase, telomeres, and associated biological processes. Methodologically driven, the telomere/telomerase research area is moving toward clinical trials of the best chemical agents. One hopes that the first clinical trials will be conducted in a manner such that if the results are negative, there will be a sufficient level of science so that a poor study design will not lead to the premature demise of this exciting area.

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