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Advances in Brief

Detection of Telomerase Inhibitors Based on G-Quadruplex Ligands by a Modified Telomeric Repeat Amplification Protocol Assay[1]

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Abstract

The telomeric repeat amplification protocol (TRAP) is commonly used to evaluate telomerase activity in tissues or cell extracts and also to determine the inhibitory properties of small molecules against telomerase. The recent discovery of G-quadruplex ligands as potent telomerase inhibitors prompted us to examine the accuracy of TRAP to be used to screen such class of molecules. Because of the specific feature of the TS primer, TRAP only allows the detection of G-quadruplex-induced telomerase inhibition after the synthesis of four TTAGGG repeats by telomerase and may thus lead to misinterpretations during screening assays. We have developed a TRAP-G4 assay that will allow the unambiguous detection of the inhibitory properties of a G-quadruplex ligand on telomerase activity and is able to discriminate them from other telomerase inhibitors.

Introduction

Human telomerase is a ribonucleoprotein composed of a catalytic subunit, human telomerase reverse transcriptase and a 451-nucleotide-long RNA (hTR), which acts as a template for the addition of the repetitive hexameric motif (5′-GGTTAG-3′) at the end of the telomeres. A number of recent studies have indicated that telomerase expression is associated with cell immortalization and tumorigenesis (1, 2). Telomerase is overexpressed in a large number of tumors, whereas it is not expressed in most somatic cells, which usually have longer telomeres. Such differential expression was the initial rationale for the evaluation of telomerase inhibitors as potential anticancer drugs (3). Different strategies have been developed to impair telomerase activity and to prevent tumor growth development, including antisense oligonucleotides and small molecule inhibitors (reviewed in Refs. 4–7).

Recently, a new class of carboxylic amide derivatives that interact with the catalytic subunit of telomerase was shown to induce progressive telomere shortening and delayed growth arrest (8). In addition, ligands of the G-quadruplex telomeric DNA structure have aroused interest recently, because it was demonstrated that these compounds were potent telomerase inhibitors in cancer cells and can cause telomere shortening and cell crisis (9). A number of reviews on G-quadruplex and their targeting by small molecules have appeared recently (5, 10, 11).

The search for telomerase inhibitors was made possible by the introduction of enzymatic assays that allow the measurement of telomerase activity in cell extracts. A PCR assay, the TRAP,[3] has been developed to measure telomerase activity (12). The original method was improved by the addition of a tag sequence at the 5′-end of the reverse primer and the use of an internal control that coamplifies with the telomerase products and allows the detection of Taq inhibitors (13–15).

The evaluation of G-quadruplex interacting agents is generally based on different techniques including temperature melting absorbance assays, fluorescence resonance energy transfer-based assays, electrophoresis analysis of dimer quadruplex formation, and dialysis equilibrium-based assays (16–18). Further analysis to establish the inhibitory properties of the compound is usually performed by using the classical TRAP assay or by primer extension with telomerase (19). Information concerning the selectivity of telomerase inhibition is essential to select pharmacological agents among G4-interacting candidates. Selectivity assays involve other DNA-interacting enzymes, such as Taq polymerase, and may be coupled with the TRAP assay thanks to an internal control (ITAS; Ref. 13).

We describe here an amplification protocol that uses an oligonucleotide primer that is susceptible to form an intramolecular G-quadruplex and is also a substrate for telomerase elongation. The protocol is suitable for studying the inhibition by G-quadruplex-interacting agents of telomerase-catalyzed reaction, and the presence of ITAS allowed us to determine the selectivity of potential inhibitors compared with Taq polymerase inhibition.

Materials and Methods

Oligonucleotides and Compounds. All oligonucleotides were synthesized and purified by Eurogentec (Belgium). Triazine derivatives were obtained from Dr. P. Mailliet (Aventis-Pharma, Vitry sur Seine, France), and their synthesis is described in patent WO 0140218. Solutions of compounds were prepared at 10 mM in DMSO and were kept at −20°C in the dark. Further dilutions were made in water.

UV Melting Experiments. All measurements were performed as described previously (17, 20, 29).

Telomerase Assay ("TRAP-G4"). Telomerase extract was prepared from A549 cells as described before (21). TRAP-G4 was performed by using a modification of the TRAP assay (12–14). PCR was performed in a final 50-μl reaction volume composed of a 45-μl reaction mix containing 20 mM Tris-HCl (pH 8.0), 50 μM deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 63 mM KCl, 1 mM EGTA, 0.005% Tween 20, 20 μg/ml BSA, 3.5 pmol of primer TSG4 (5′-GGGATTGGGATTGGGATTGGGTT-3′), 18 pmol of primer TS (5′-AATCCGTCGAGCAGAGTT-3′), 22.5 pmol of primer CXext (5′-GTGCCCTTACCCTTACCCTTACCCTAA-3′), 7.5 pmol of primer NT (5′-ATCGCTTCTCGGCCTTTT-3′), 0.01 amol of TSNT internal control (5′-ATTCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3′), 2.5 units of Taq DNA polymerase (DyNAzyme II DNA polymerase; Ozyme), and 100 ng of telomerase. Compounds or distilled water were added under a volume of 5 μl. PCR were performed in an Eppendorf Mastercycler equipped with a hot lid and incubated for 15 min at 30°C, 1 min at 90°C followed by 30 cycles: 30 s 92°C, 30 s 52°C, and 30 s 72°C. After amplification, 8 μl of loading buffer containing 20% sucrose, 5× Tris-Borate-EDTA buffer, 0.2% bromphenol blue, and 0.2% xylene cyanol were added to the reaction. A 15-μl aliquot was loaded onto a 12% nondenaturing acrylamide gel (19:1) in 1× TBE and

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[3] The abbreviations used are: TRAP, telomeric repeat amplification protocol; ITAS, internal telomerase assay standard.

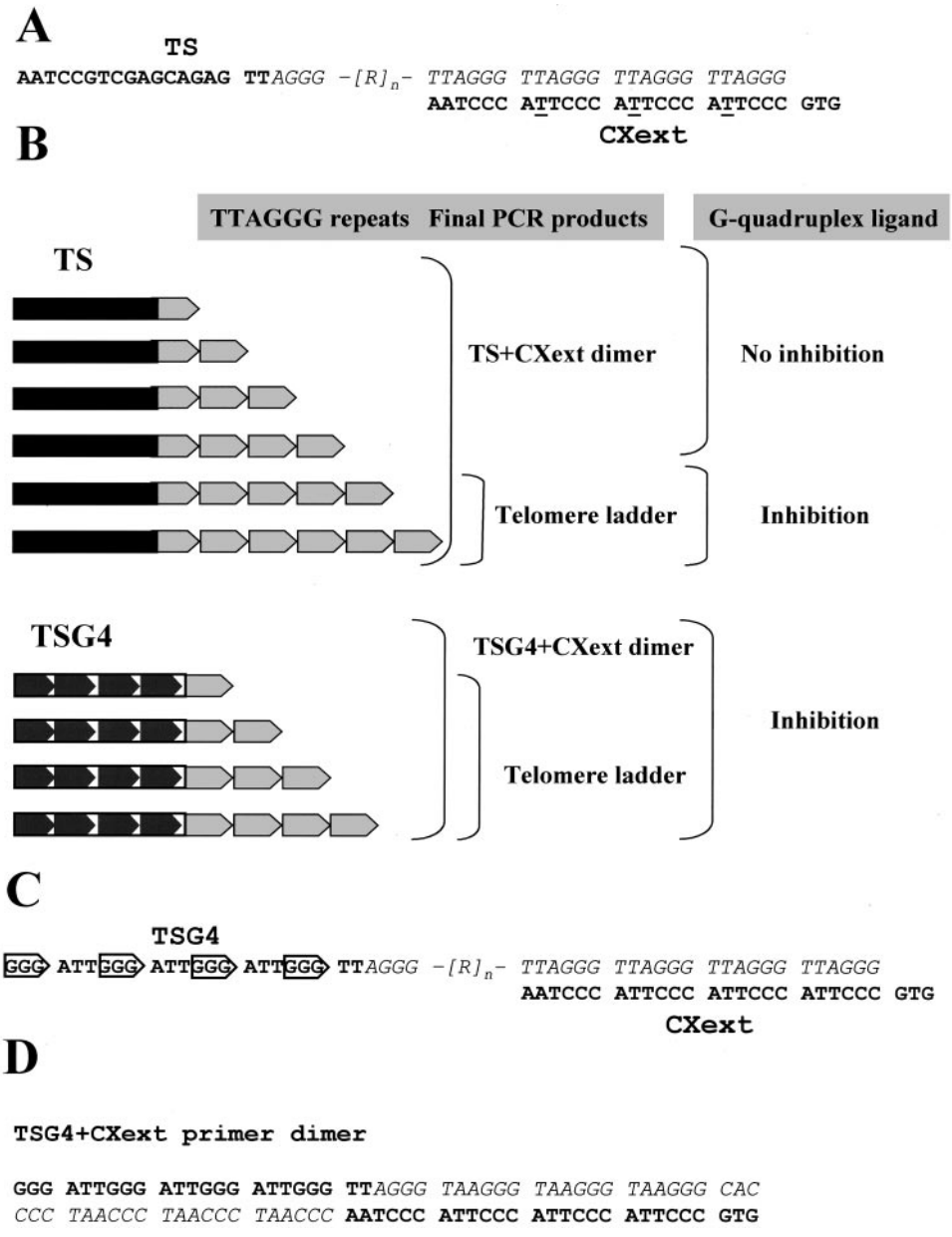

Fig. 1. *A*, sequence of TS primer. Telomerase elongates the TS primer, adding TTAGGG repeats. During PCR, CXext hybridizes to the 5′ end of the repeats in subsequent PCR steps; TS and CXext primers are shown in *bold*. Mismatched nucleotides are *underlined*. $[R]_n$ corresponds to *n* repetitions of TTAGGG. *B*, schematic representation of the telomerase products (TTAGGG repeats) extended from: the TS primer in TRAP (*upper part*) or from the TSG4 primer in TRAP-G4 (*lower part*). When 0–4 TTAGGG repeats are added to TS by telomerase, final PCR products correspond to TS+CXext dimer; a G-quadruplex ligand should not have an inhibitory effect on their formation. When >4 TTAGGG repeats are added by telomerase, final PCR products correspond to TS+CXext dimer and telomere ladder. A G-quadruplex ligand should inhibit telomere ladder formation. In the presence of TSG4, a quadruplex ligand should inhibit both TSG4+CXext dimer and telomere ladder formation. *C*, sequence of TSG4 primer. Telomerase elongates the TSG4 primer, adding TTAGGG repeats. CXext hybridize to the 5′-end of the repeats in subsequent PCR steps. TSG4 and CXext primers are shown in *bold*. Mismatched nucleotides are *underlined*. $[R]_n$ corresponds to *n* repetitions of TTAGGG. *D*, minimal overlap leading to TSG4+CXext primer dimer formation that yields elongated TSG4 with four added repeats.

electrophoresed at 200 V for 1 h using Xcell Surelock Minicell apparatus (Novex). Gels were stained with 1× SYBR Green I (Roche) and digitalized by a CCD camera (Bioprint). As indicated, classical TRAP was performed in the same buffer and PCR conditions but without TSG4 and in the presence of primer TS and CXext (15 pmol), with or without ITAS primers NT (7.5 pmol) and TSNT (0.01 amol).

Results and Discussion

G-quadruplex-interacting agents block telomerase extension through the stabilization of a G-quadruplex structure that further inhibits the translocation step of the enzyme (22–24). Because the formation of an intramolecular G-quadruplex requires at least four TTAGGG repeats in the portion of the primer that is not base-paired to the hTR RNA template, inhibition of the telomerase extension would be detectable after the synthesis of four TTAGGG repeats.

The TS oligonucleotide used in the classical TRAP assay does not contain the guanine repeats characteristic of the telomeric sequence (18-mer sequence shown in bold in Fig. 1*A*; Ref. 12). Therefore, inhibition of telomerase by G-quadruplex ligands in the TRAP assay will only be detectable after the synthesis of at least four TTAGGG repeats (Fig. 1*B*). Further PCR amplification of the telomerase products with the reverse primer CXext will generate a telomere ladder that reflects telomerase activity but also the minimal TS+CXext 43-mer dimer product. The latter represents either telomerase extension of 1–4 TTAGGG repeats or dimer formation between TS and CXext primer, as already reported (14).

Triazine derivatives were recently reported to display potent inhibition of the telomerase function by stabilizing G4-DNA structure (9). In the presence of the triazine derivative 12459, the TRAP assay revealed a dose-dependent inhibition of the telomere ladder, starting at 0.5 μM and completing at 5 μM (Fig. 2*A*). However, no inhibition of the 43-mer TS+CXext dimer could be evidenced at these concentrations, in agreement with the consideration that inhibition would be detectable after the synthesis of the fourth TTAGGG repeat. Inhibition of the TS+CXext dimer is detected in the presence of 10 μM 12459, whereas inhibition of ITAS occurred at 20 μM. Indeed, high concentrations of 12459 may lead to non G-quadruplex-related effects block-

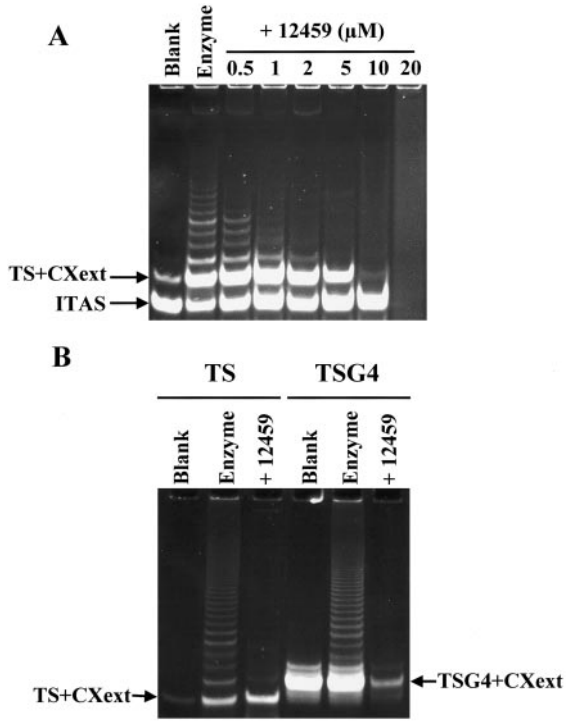

Fig. 2. *A*, inhibition of telomerase activity by a G-quadruplex ligand in a classical TRAP assay. Increasing concentrations of 12459 (0.5–20 μM) were added to 100 ng of telomerase extract in a TRAP assay containing primers TS and CXext (15 pmol), primer NT (7.5 pmol), and TSNT (0.01 amol) internal standard (ITAS). *Enzyme*, the telomerase extract with no added compound. *Blank*, TRAP without telomerase extract. Inhibition of telomere ladder started at 0.5 μM, whereas complete inhibition of TS+CXext and ITAS was observed at 10 and 20 μM, respectively. *B*, comparison between primer TS and TSG4 in the TRAP assay. One hundred ng of telomerase extract alone (*Enzyme*) or in the presence of 1 μM 12459 were assayed in TRAP assay with either TS or TSG4 oligonucleotide (15 pmol) and CXext primer (15 pmol). The ITAS primers (NT and TSNT) were omitted in the reaction. *Blank*, TRAP without telomerase extract. TS+CXext or TSG4+CXext bands corresponding to minimal dimer PCR products between reverse and forward primers are indicated by *arrows*.

ing the TS+CXext dimer formation. Alternatively, a partial extension of TS by telomerase (<4 TTAGGG repeats) might possibly generate and stabilize dimer G2-quadruplexes in the presence of 12459 that further block PCR amplification.

The peculiar features of the G-quadruplex ligand inhibition on TRAP assay may therefore cause important misinterpretations of the results when gel analysis is replaced by other technologies to reveal the amplified products, such as scintillation proximity or ELISA (25, 26). In these cases, TS+CXext dimer formation may be wrongly included for the calculation of the telomerase products, and the IC_{50} will underestimate the inhibitory property and the potential selectivity of the compound. For example, in the experiment presented in Fig. 2*A*, the IC_{50} for 12459 will reach 7.5 μM instead of 0.5 μM when TS+CXext is included in the calculation.

The modification of the TS primer in the TRAP assay by an oligonucleotide susceptible to form an intramolecular G-quadruplex and also to be extended by telomerase is a possible alternative to overcome these limitations in the analysis of G-quadruplex-interacting agents. Telomerase can add TTAGGG repeats to a variety of nontelomeric sequences *in vitro* (27, 28). Oligonucleotide TSG4 was designed to mimic the GGG tracts of the telomeric sequence and to minimize the possibilities of hybridization with CXext. For that purpose, the telomeric triplet TTA was replaced by ATT (Fig. 1*C*). TSG4 is elongated by telomerase and gives a classical telomeric ladder when used in the TRAP assay instead of TS primer (Fig. 2*B*). In the absence of telomerase, TSG4 forms a 53-mer band that corresponds to the TSG4+CXext dimer (Fig. 1*D*). In the presence of 1 μM 12459, complete TRAP inhibition, including the formation of both telomerase ladders and TSG4+CXext dimer, is achievable (compare with the classical TRAP shown in Fig. 2*B*).

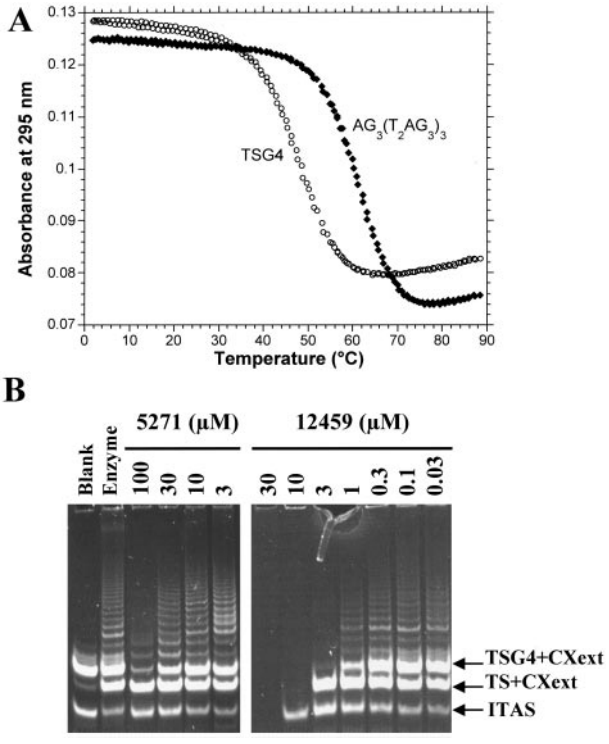

Fig. 3. *A*, UV-melting behavior of TSG4 and 22AG oligonucleotides in 0.063 M KCl. Denaturation of the Quadruplex is shown by a decrease in the absorbance at 295 nm. *B*, inhibition of telomerase activity by G-quadruplex ligands in the TRAP-G4 assay. Triazines 5271 or 12459, at the indicated concentrations, were added to 100 ng of telomerase extract in the conditions of the TRAP-G4 assay with primers TSG4 (3.5 pmol), TS (18 pmol), CXext (22.5 pmol), NT (3.5 pmol), and TSNT (0.01 amol). *Enzyme*, telomerase extract without compound. *Blank*, TRAP-G4 assay without telomerase extract. *Arrows*, positions of TSG4+CXext, TS+CXext, and ITAS PCR products. G-quadruplex ligands inhibit telomere ladder and TSG4+CXext dimer formation at lower concentrations than TS+CXext and ITAS formations.

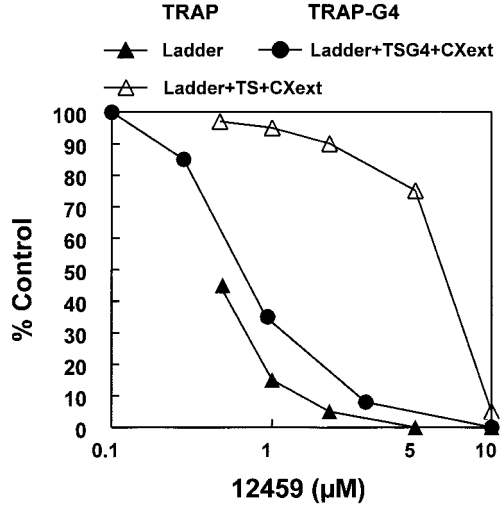

Fig. 4. Comparison between "classical" TRAP and TRAP-G4 for 12459 inhibition. Inhibition of telomerase ladder inhibition in TRAP assay (♦) and telomerase ladder+TSG4+CXext dimer inhibition in TRAP-G4 assay (●) were achieved at very similar 12459 concentrations. In contrast, TRAP assay underestimate the potency of 12459 when telomerase ladder and TS+CXext dimer are included in the calculation (△). SYBR Green fluorescent emission from experiments presented in Figs. 2*B* and 3*B* were digitalized by a CCD camera under nonsaturating conditions, and results were expressed as a percentage of the signal measured in enzyme untreated reactions (control).

Under these conditions, TRAP inhibition certainly results from a stabilization of the TSG4 primer into an intramolecular G-quadruplex structure. Because TSG4 presents differences with the telomeric DNA, we have determined whether this oligonucleotide could form a G-quadruplex under the conditions of the TRAP. The oligonucleotide structure is monitored according to the temperature variation by UV spectroscopy at 295 nm (29) in 0.063 M KCl (Fig 3*A*). A melting point of 48.5°C was determined, which indicates that the modification of TTA repeats into ATT significantly destabilizes the G-quadruplex, as compared with 22AG (*Tm*, 61°C). Therefore, the KCl concentration used in the TRAP assay allows the formation of G-quadruplex by TSG4. Nevertheless, this quadruplex is less stable than the telomeric 22AG ($\Delta G°_{(30°C)}$, 2.8 kcal/mol; which corresponds to a 43-fold difference in affinity constant). As a consequence, at 30°C and in the absence of a quadruplex-stabilizing molecule, TSG4 may be efficiently unfolded by telomerase and is efficiently extended (see Fig. 2*B, TSG4 Lane Enzyme*). Upon addition of 12459, the stability of the TSG4 strongly increases, and TSG4 may not prime the enzyme nor subsequent PCR amplification to form the TSG4+CXext dimer form.

The inclusion in the assay of both TS and TSG4 primers, together with the internal ITAS, allowed us to discriminate between G4-based telomerase and catalytic inhibition of the enzyme. A typical experiment for such assay, called TRAP-G4, is presented for 12459 and 5271, a nearly inactive triazine derivative (Fig. 3*B*; Ref. 9). G-quadruplex-related inhibitory properties (IC_{50G4}) could be quantified by integrating the fluorescent signal from telomeric ladders and TSG4+CXext, whereas catalytically related telomerase (IC_{50Telo}) and Taq polymerase (IC_{50Taq}) inhibitory properties were obtained by integrating TS+CXext dimer and ITAS, respectively. For 12459, IC_{50G4} was equal to 0.6 μM, a concentration far below that necessary to block catalytic activity of telomerase (IC_{50Telo}, 6 μM) and Taq polymerase (IC_{50Taq}, 15 μM). In the presence of 5271, the TRAP-G4 assay indicates that no telomerase or Taq polymerase inhibition was achieved at 100 μM, whereas a very weak G4-related telomerase inhibitory activity was found (IC_{50G4}, 60 μM). These results were in agreement with our previous findings of G-quadruplex stabilization for these derivatives (9).

Although the nature of the G-quadruplex structure introduced in the TSG4 oligonucleotide is different from the telomeric quadruplex, our results indicate that telomere ladder inhibition in a classical TRAP was achieved at a very similar 12459 concentration than telomere ladder and TSG4+CXext inhibition in the TRAP-G4 assay (Fig. 4). Thus, 12459 does not appear to distinguish between these two quadruplexes, a finding that is consistent with results obtained by competitive equilibrium dialysis for this compound with other G-quadruplex species.[4]

In summary, we have introduced a new TRAP-G4 that first includes a G-quadruplex sequence into the primer elongated by telomerase and allows us to accurately measure the inhibitory properties of G-quadruplex ligands on telomerase activity. In principle, the TSG4 primer may also be used in a radioactive-based assay. The use of the TSG4 primer in an automated TRAP-G4 assay with different fluorescently labeled oligonucleotides will allow the development of a rapid and powerful tool to screen for new G-quadruplex-interacting agents with telomerase-inhibitory properties.

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[4] J. L. Mergny, unpublished results.

References

1. Holt, S. E., and Shay, J. W. Role of telomerase in cellular proliferation and cancer. J. Cell. Physiol., *180:* 10–18, 1999.
2. McEachern, M. J., Krauskopf, A., and Blackburn, E. H. Telomeres and their control. Annu. Rev. Genet., *34:* 331–358, 2000.
3. Sharma, S., Raymond, E., Soda, H., Sun, D., Hilsenbeck, S. G., Sharma, A., Izbicka, E., Windle, B., and Von Hoff, D. D. Preclinical and clinical strategies for development of telomerase and telomere inhibitors. Ann. Oncol., *8:* 1063–1074, 1997.
4. Lavelle, F., Riou, J. F., Laoui, A., and Mailliet, P. Telomerase: a therapeutic target for the third millennium? Crit. Rev. Oncol. Hematol., *34:* 111–126, 2000.
5. Perry, P. J., Arnold, J. R., and Jenkins, T. C. Telomerase inhibitors for the treatment of cancer: the current perspective. Expert Opin. Investig. Drugs, *10:* 2141–2156, 2001.
6. White, L. K., Wright, W. E., and Shay, J. W. Telomerase inhibitors. Trends Biotechnol., *19:* 114–120, 2001.
7. Mergny, J. L., Riou, J. F., Mailliet, P., Teulade-Fichou, M. P., and Gilson, E. Natural and pharmacological regulation of telomerase. Nucleic Acids Res., *30:* 839–865, 2002.
8. Damm, K., Hemmann, U., Garin-Chesa, P., Hauel, N., Kauffmann, I., Priepke, H., Niestroj, C., Daiber, C., Enenkel, B., Guilliard, B., Lauritsch, I., Muller, E., Pascolo, E., Sauter, G., Pantic, M., Martens, U. M., Wenz, C., Lingner, J., Kraut, N., Rettig, W. J., and Schnapp, A. A highly selective telomerase inhibitor limiting human cancer cell proliferation. EMBO J., *20:* 6958–6968, 2001.
9. Riou, J. F., Guittat, L., Mailliet, P., Laoui, A., Renou, E., Petitgenet, O., Megnin-Chanet, F., Helene, C., and Mergny, J. L. Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. Proc. Natl. Acad. Sci. USA, *99:* 2672–2677, 2002.
10. Raymond, E., Soria, J. C., Izbicka, E., Boussin, F., Hurley, L., and Von Hoff, D. D. DNA G-quadruplexes, telomere-specific proteins and telomere-associated enzymes as potential targets for new anticancer drugs. Investig. New Drugs, *18:* 123–137, 2000.
11. Hurley, L. H., Wheelhouse, R. T., Sun, D., Kerwin, S. M., Salazar, M., Fedoroff, O. Y., Han, F. X., Han, H., Izbicka, E., and Von Hoff, D. D. G-quadruplexes as targets for drug design. Pharmacol. Ther., *85:* 141–158, 2000.
12. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. Specific association of human telomerase activity with immortal cells and cancer. Science (Wash. DC), *266:* 2011–2015, 1994.
13. Kim, N. W., and Wu, F. Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). Nucleic Acids Res., *25:* 2595–2597, 1997.
14. Krupp, G., Kuhne, K., Tamm, S., Klapper, W., Heidorn, K., Rott, A., and Parwaresch, R. Molecular basis of artifacts in the detection of telomerase activity and a modified primer for a more robust "TRAP" assay. Nucleic Acids Res., *25:* 919–921, 1997.
15. Wright, W. E., Shay, J. W., and Piatyszek, M. A. Modifications of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. Nucleic Acids Res., *23:* 3794–3795, 1995.
16. Han, H., Cliff, C. L., and Hurley, L. H. Accelerated assembly of G-quadruplex structures by a small molecule. Biochemistry, *38:* 6981–6986, 1999.
17. Mergny, J. L., and Maurizot, J. C. Fluorescence resonance energy transfer as a probe for G-quartet formation by a telomeric repeat. Chembiochem. Eur. J. Chem. Biol., *2:* 124–132, 2001.
18. Ren, J., and Chaires, J. B. Sequence and structural selectivity of nucleic acid binding ligands. Biochemistry, *38:* 16067–16075, 1999.
19. Cathers, B. E., Sun, D., and Hurley, L. H. Accurate determination of quadruplex binding affinity and potency of G-quadruplex-interactive telomerase inhibitors by use of a telomerase extension assay requires varying the primer concentration. Anticancer Drug Des., *14:* 367–372, 1999.
20. Mergny, J. L., Mailliet, P., Lavelle, F., Riou, J. F., Laoui, A., and Helene, C. The development of telomerase inhibitors: the G-quartet approach. Anticancer Drug Des., *14:* 327–339, 1999.
21. Fu, W., Begley, J. G., Killen, M. W., and Mattson, M. P. Anti-apoptotic role of telomerase in pheochromocytoma cells. J. Biol. Chem., *274:* 7264–7271, 1999.
22. Zahler, A. M., Williamson, J. R., Cech, T. R., and Prescott, D. M. Inhibition of telomerase by G-quartet DNA structures, Nature (Lond.), *350:* 718–720, 1991.
23. Fletcher, T. M., Sun, D., Salazar, M., and Hurley, L. H. Effect of DNA secondary structure on human telomerase activity. Biochemistry, *37:* 5536–5541, 1998.
24. Salazar, M., Thompson, B. D., Kerwin, S. M., and Hurley, L. H. Thermally induced DNA. RNA hybrid to G-quadruplex transitions: possible implications for telomere synthesis by telomerase. Biochemistry, *35:* 16110–16115, 1996.
25. Savoysky, E., Akamatsu, K., Tsuchiya, M., and Yamazaki, T. Detection of telomerase activity by combination of TRAP method and scintillation proximity assay (SPA). Nucleic Acids Res., *24:* 1175–1176, 1996.
26. Hoos, A., Hepp, H. H., Kaul, S., Ahlert, T., Bastert, G., and Wallwiener, D. Telomerase activity correlates with tumor aggressiveness and reflects therapy effect in breast cancer. Int. J. Cancer, *79:* 8–12, 1998.
27. Harrington, L. A., and Greider, C. W. Telomerase primer specificity and chromosome healing. Nature (Lond.), *353:* 451–454, 1991.
28. Morin, G. B. Recognition of a chromosome truncation site associated with α-thalassaemia by human telomerase. Nature (Lond.), *353:* 454–456, 1991.
29. Mergny, J. L., Phan, A. T., and Lacroix, L. Following G-quartet formation by UV-spectroscopy. FEBS Lett., *435:* 74–78, 1998.

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