G-Quadruplex Interacting Agents Targeting the Telomeric G-Overhang Are More than Simple Telomerase Inhibitors

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Abstract: The extremities of chromosomes end at telomeres in a G-rich single stranded overhang that may adopt peculiar structures such as T-loop and G-quadruplex. G-quadruplex is a poor substrate for telomerase activity and different classes of small molecule ligands that selectively stabilize this structure and inhibit telomerase activity have been selected by screening or synthesized by oriented chemistry. These ligands differ from catalytic inhibitors of telomerase by several points that were discussed in the present review, with a special emphasis on their biological activity as potential antitumor agents.

TELOMERE STRUCTURE AT CHROMOSOME ENDS

Eukaryotic chromosomes end with a specific nucleoprotein structure called the telomere [1, 2]. Telomere associated proteins hide telomere DNA from being recognized as DNA breaks and allow cells to distinguish between a normal telomere end or the result of double-stranded DNA breaks [2]. Telomere contributes to the spatial and functional organisation of chromosomes within the nucleus and provides a mean for complete replication of the linear end of chromosomes [1].

Telomere is of particular interest because of its function to maintain the indefinite proliferation of cells [3]. In human somatic cells, telomere length decreases at each round of division and cellular mechanisms that counter act this degradation are able to confer indefinite proliferation potential. Two classes of mechanisms have been described in human tumor cells that allowed the maintenance of telomere length. (i) A specialized enzyme called telomerase is able to copy, as a reverse transcriptase, the short TTAGGG motif at the end of telomere. Telomerase is composed of a catalytic subunit, hTERT, associated with a RNA containing the template of the telomere repeat unit, hTR [4]. Telomerase is overexpressed in a large number of tumors (about 85%) and is involved in the capping of telomere ends [5]. (ii) In about 15% of human tumors, telomerase activity is lacking and the telomere shortening of cancer cells is kept by recombination mechanisms between telomeres, a mechanism known as Alternative Lengthening of Telomere (ALT) [6, 7]. Telomere length is heterogeneous in human tumors, varying from long and heterogeneous telomeres in ALT cells and shorter size (between 4 to 7 kb) in telomerase positive cells. Telomere length reflects the homeostasis between telomere elongation and telomere capping that protects from recognition by the DNA damaging pathway: short telomeres required highest capping [2]. Telomerase itself, together with other telomere binding proteins such as TRF2 and Pot1, is involved in the control of telomere capping [2, 8, 9]. Uncapping of telomeres resulted in genetic instability and disorder at mitosis.

Telomere ends in a 3’ single-stranded overhang averaging 130-210 bases in length that may be involved in different DNA conformations such as T-loops or G-quadruplexes. Single-stranded overhangs, also called as G-overhang exist during most of the cell cycle and are present on all chromosomal ends [1, 10-12]. T-loops are created through the strand invasion of the 3’ telomeric overhang into the duplex part of the telomere and are thought to represent a strategy to protect chromosome end fusion by an overhang sequestration mechanism [9]. Due to the repetition of guanines, the G-overhang is prone to quadruplex formation in which 4 blocks of repeated guanines are engaged into 3 adjacent quartets that involves 4 guanines stabilized by Hoogsteen bonds [13, 14]. The G-overhang can fold into at least two different intramolecular quadruplexes that differed by the position of the adjacent loop regions [15-17]. The intramolecular telomeric quadruplex is quite stable under physiological pH and salt conditions [18, 19].

SEARCH FOR G-QUADRUPLEX LIGANDS INHIBITORS OF TELOMERASE

The telomerase enzyme is inactive (or less active) in somatic cells, as compared to cancer cells [20] and thus presents a new interesting and very specific target for anticancer drug development. Compounds that targeted the reverse transcriptase subunit of telomerase, its RNA component or the telomeric DNA substrate have been reported [14, 21]. Optimal telomerase activity requires the non-folded single-stranded telomere overhang and G-quadruplex formation has been shown to inhibit telomerase elongation in vitro [22]. Therefore, ligands that selectively bind to G-quadruplex structure may modulate telomerase activity [23].

Several classes of small molecules that bind to telomeric G-quadruplex DNA and inhibit telomerase activity have been described, (see Fig. 1) such as porphyrins [24, 25], perylenes [26], amidoanthracene-9,10-diones [27], 2,7-disubstituted amidofluorenones [28], acridines [29, 30],

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1568-0118/04 $45.00+.00 © 2004 Bentham Science Publishers Ltd.
ethidium derivatives [31, 32], disubstituted triazines [14], fluoroquinanthroxazines [33] indoloquinolines [34], dibenzophenanthrolines [35], bisquinacridines [36], pentacyclic acridinium [37], telomestatin [38, 39] and more recently 2,6-pyridin-dicarboxamide derivatives [40] (for a review see [41, 42]).

Due to the peculiar features of the quadruplex structure, as compared to classical double-stranded B-DNA, it could be expected a selective recognition of telomeric G-quadruplex by small molecule ligands [42]. Some partial selectivity was obtained with triazine [43] or ethidium derivatives [32] and was significantly enhanced with the natural product telomes-
Different approaches have been used to identify these G-quadruplex ligands. They could be divided in two classes (i) fluorescence assays and (ii) enzymatic assays.

(i) FRET melting experiments are often used to probe the secondary structure of oligonucleotides mimicking the telomeric repeat in which a donor and a fluorescence acceptor are attached at one end of the oligonucleotide. The melting of the G-quadruplex is monitored in the presence of the ligand and G-quadruplex interacting G-quadruplex ligands have been shown to have telomerase inhibitory properties in vitro. This assay called TRAP (Telomeric Repeat Amplification Protocol) is a PCR based assay that amplified the telomere product synthesized by telomerase [47]. The inclusion of a primer able to form a G-quadruplex structure as a template for telomerase in the TRAP-G4 assay allowed to determine the inhibitory properties of ligands against telomerase more accurately [48]. A good correlation was found between telomerase inhibition and G-quadruplex stabilization measured by FRET among series of more than a hundred derivatives tested so far [35].

DIFFERENCE BETWEEN TELOMERE TARGETING AGENTS AND INHIBITORS OF TELOMERASE

Targeting the substrate of an enzyme is an original strategy to inhibit its activity. There are fundamental differences with the targeting of telomere G-overhang and the targeting of telomerase subunits (hTERT and hTR). Telomeres exist in the absence of telomerase activity and played at least one fundamental function in ALT cells: the protection (capping) of chromosome ends [49]. G-quadruplex ligands might then have an effect against ALT tumor cells in contrast to catalytic inhibitors but also on normal cells that do not maintain telomere length, leading to potential undesired toxicity. Since genetic instability and telomere alterations are hallmarks of cancer cells, it is also assumed that telomere capping greatly differs between normal and cancer cells, leading to a potential therapeutic index in the clinical use of these agents.

According to the initial paradigm for telomerase inhibitors, these agents should not affect growth rate initially but induce progressive telomere shortening. A decreased proliferation should be observed only when telomeres reach a critically short length. Therefore, the delay necessary for the antiproliferative effect should depend on initial telomere length. This paradigm has been verified by several key experiments using hTERT dominant negatives or with catalytic inhibitors of the enzyme [50, 51]. For G-quadruplex ligands this paradigm is partially true, these ligands presented classical telomerase inhibitory properties at low dosages but presented a much more rapid action at higher concentrations.

For example, long term treatment of human A549 lung adenocarcinoma tumor cells was performed with subtoxic concentrations of two triazine derivatives, 115405 and 12459 [43]. A delayed growth arrest that depends on the initial telomere length was observed. This growth arrest is associated with telomere erosion and the appearance of a senescent-like cell phenotype (large morphology and expression of β-galactosidase activity).

Nonetheless, recent experiments suggest that inhibition of telomerase activity induces dramatic and rapid consequences on cell viability. Short-term and massive apoptosis may result from interference with telomere function when either hTERT or hTR are modified by mutations [52]. The G-quadruplex-stabilizing compounds are also active as antiproliferative agents on a panel of 13 human cancer cell lines and 2 immortalized human cell lines [43].

Apoptotic and short-term response of leukaemia cell lines were also reported during telomestatin treatment [53]. Since all ligands previously reported to stabilize telomeric G-quadruplex and to inhibit telomerase activity also display potent activities against other G-quadruplexes such as c-myc it may be argued that the short-term inhibition of cell proliferation might be due to the action of the ligands against other quadruplexes controlling the function of a gene implicated in the cell proliferation [54]. It has also been reported that the antiproliferative effect of G4 ligands is independent on the presence of telomerase activity. Overexpression of hTERT or a dominant negative of hTERT in the A549 human lung carcinoma cell line did not modify the antiproliferative effect of the ligand [55]. Moreover, highly selective G4 ligands of the 2,6 pyridine-dicarboxamide series were recently found to induce a cell cycle arrest only after several rounds of replication in two telomerase-positive glioma cell lines with short telomeres, T98G and CB193 1. This cell cycle arrest was linked to a block during S phase and was followed by a massive induction of apoptosis.

In addition, TMPyP4 and telomestatin were also found to be active to induce long-term senescence and 12459 ligand to induce short-term antiproliferative activity in ALT tumor cell lines [43, 45]. These results might suggest that the direct target of these ligands is rather telomere than telomerase activity. Increasing evidences indicate that the capping of the single-strand telomeric overhang might be important to control senescence [56, 57] and the hypothesis that these ligands provoke uncapping of the telomeric overhang has emerged. Our group has now evidence that G-quadruplex ligands such as telomestatin or 2,6 pyridine-dicarboxylate derivatives efficiently impair the single-stranded conformation of the G-overhang. A tight and specific interaction of telomestatin with telomeric overhang compatible with the formation of stable G-quadruplex was observed and prolonged treatment of tumor cells with this ligand resulted in a strong decrease of the overhang signal that correlated with the onset of replicative senescence, thus indicating that telomeric G-overhang is the real intracellular target for the telomeres.
action of these compounds. A recent report indicates that TRF2 is essential for the protection of the telomeric overhang and prevents the action of the excision repair nuclease ERCC1/XPF that participates in the overhang removal [58]. The alteration of the G-overhang conformation by G-quadruplex ligands is therefore proposed to alter its capping by essential factors, such as TRF2, thus provoking its rapid degradation and subsequent cell cycle arrest or apoptosis.

The final question remains the selectivity of these ligands towards normal cells. Telomestatin that was reported to induce senescence and/or apoptosis against different tumor cell types displays an interesting selectivity towards normal CFU-GM progenitor [53]. Identical results were found with the triservative 12459 and with new derivatives from the 2,6 pyridin-dicarboxylate series that were found fold less potent for short-term exposure against normal cells in culture than against tumour cells. To date, no antitumor agent in clinic that targets directly or indirectly the DNA duplex (intercalating agent, topoisomerase inhibitors, platin...) presented any significant selectivity between dividing normal cells and tumor cell lines but displayed in vivo a more or less narrow window for selectivity called “therapeutic index”. This opens the door to an interesting situation in the light of the future clinical development of this new class of antitumor agent.

ACKNOWLEDGEMENTS

This work was financed by the “Ministère de la Recherche” and an ARC grant (#4691).

REFERENCES


