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Cancer

Telomerase as a therapeutic target in cancer

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Advances in chromosome dynamics have increased our understanding of the significant role of telomeres and telomerase in cancer. Telomerase is expressed in almost all cancer cells but is inactive in most normal somatic cells. Therefore, telomerase is an important target for the design of therapeutic agents that might have minimal side effects. Herein, we evaluate current approaches to telomerase-targeted therapy, discuss the benefits and disadvantages, and speculate on the future direction of telomerase inhibitors as cancer therapeutics.

Introduction

Cancer has recently overtaken heart disease as the primary cause of death in people in the United States aged less than 85 years (98% of the population). Most current chemotherapeutics have significant side effects for cancer patients owing to their detrimental effects on normal cells; therefore, a major challenge is finding molecular targets that are more specific to tumors and not prevalent in normal cells.

Telomerase is expressed in almost all human cancers but is inactive in most normal cells [1]. This distinction provides an attractive target for the design of inhibitors for the treatment of cancer, with the hope of little to no toxic side effects to normal cells, which do not express telomerase.

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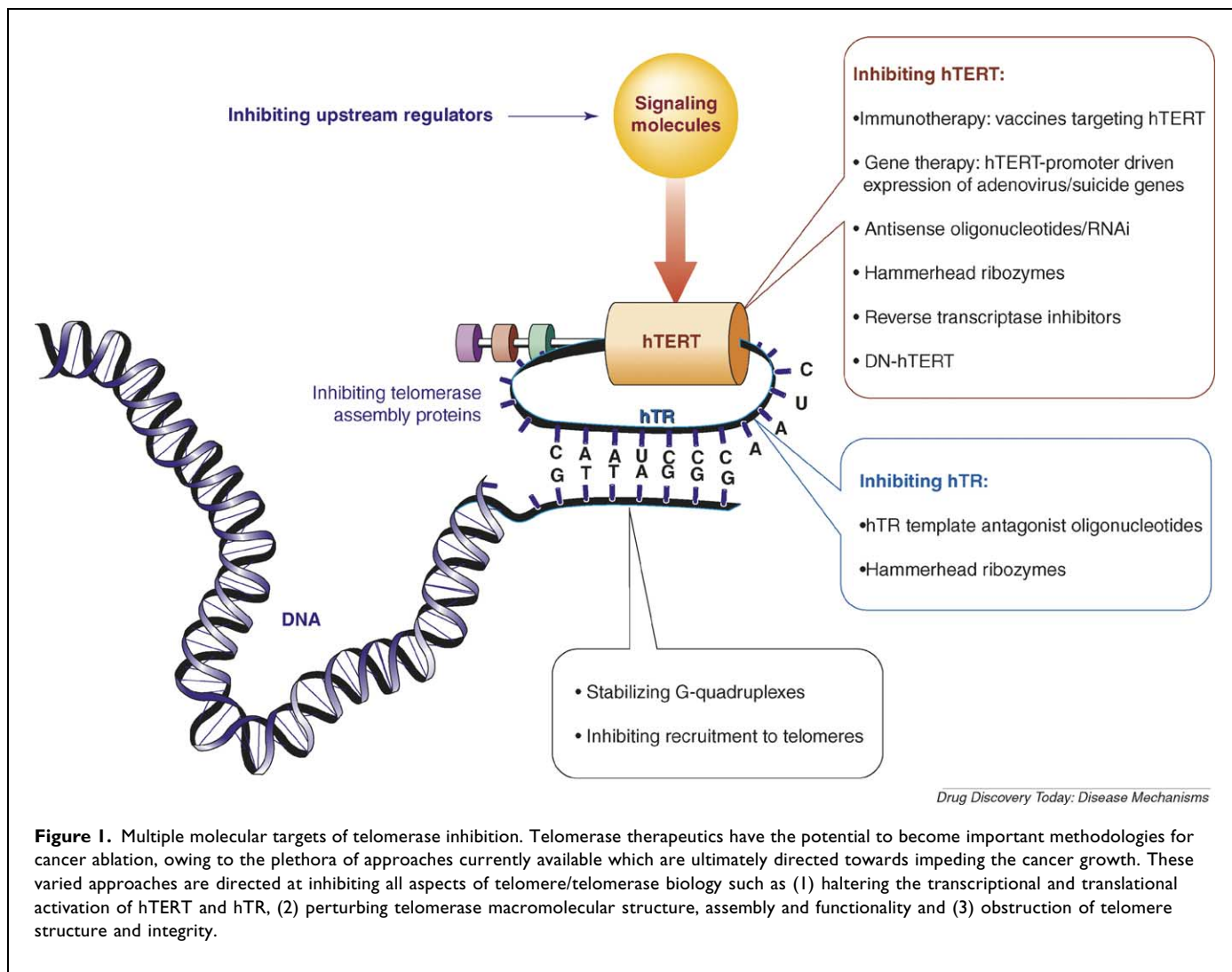
Telomeres and telomerase

Telomeres consist of tandem TTAGGG repeats that cap and protect the chromosome ends. Telomeres shorten 50–200 bp with each cell division resulting from incomplete DNA replication of the lagging strand and other end-processing events, and this shortening can be overcome by the expression of telomerase [1]. Telomerase is a ribonucleoprotein complex containing two essential components, a telomerase reverse transcriptase catalytic subunit (TERT, Gene ID 7015) and a telomerase RNA template (TR or TERC, Gene ID 7012) [1]. In cells that do not express telomerase, progressive shortening of the telomere occurs with each cell division that ultimately results in cellular (replicative) senescence [1]. Conversely, cancer cells have active telomerase that maintain telomere length, providing those cells with unlimited cell division [1].

Telomerase and cancer

Telomerase activity is present in most cancers and often correlates with the acquisition of a more malignant phenotype [2]. Telomere length is also typically shorter in tumor cells when compared to adjacent noncancerous cells [1]. A therapeutic window exists in which tumor cells might be efficiently targeted, whereas normal telomerase-expressing cells, such as stem cells and proliferating lymphocytes, might remain unaffected as a result of their longer telomere lengths.

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Drug Discovery Today: Disease Mechanisms

Numerous telomerase inhibitor strategies exist (Fig. 1, Table 1); which are the focus of this review.

Telomerase inhibitors

Immunotherapy: vaccines targeting telomerase

Human hTERT-specific epitopes are expressed on cancer cells but not on normal cells [3]. Telomerase (hTERT) is thus regarded as a universal tumor antigen owing to its expression in almost all cancers. Phase I clinical trials (Table 1) demonstrate that most patients with advanced breast [4] or prostate carcinoma [3] have induced hTERT-specific cytotoxic T lymphocytes (CTLs) following vaccination to mobilize dendritic cells that were pulsed with hTERT peptide or telomerase RNA. Importantly, there were no significant toxic side effects [3].

Gene therapy

The use of hTERT promoter-driven expression of oncolytic adenovirus and/or suicide genes is another approach that restricts the expression to the tumor. The hTERT promoter-driven expression of TRAIL (Gene ID 8743), FADD (Gene ID

8772), caspase-6 (CASP6, Gene ID 839), caspase-8 (CASP8, Gene ID 841), or Bax (Gene ID, 581) selectively induces apoptosis in telomerase-positive tumor cells, whereas no apoptosis occurs in telomerase-negative cells; intratumoral injection of these constructs into subcutaneous tumors in mice results in a significant decrease in the tumor size [1,5]. An adenoviral vector, in which the hTERT promoter drives the expression of adenoviral E1A and E1B genes, causes death of cancer cells and significant inhibition of human lung, liver and prostate tumor growth [6,7]. These studies show promise in the ability of hTERT promoter-driven expression of suicide genes or oncolytic adenoviruses to effectively and selectively kill a wide variety of cancer cells while sparing normal telomerase-negative cells.

Oligonucleotide approaches

Oligodeoxynucleotides complementary to hTERT mRNA can lead to the rapid cell death of the tumor cells [8]. By contrast, another report found only a modest inhibition of telomerase activity in prostate cancer cells, with no significant changes

Table 1. Targets and related therapies

Strategic target	Expected outcome of intervention at target	Who is working on the target	Therapies in trial
hTERT vaccine (TVAX)	Cancer remission/prevention of relapse	Robert Vonderheide, University Penn Medical (http://www.med.upenn.edu/) Geron Corporation (http://www.geron.com/) and Johannes Vieweg, Duke University Medical Center (http://www.mc.duke.edu/) Gustav Gaudernack, Norwegian Radium Hospital (http://www.radium.no/)	Yes Yes Yes
hTR/hTERC oligonucleotide	Telomerase enzyme inhibitor, telomere shortening	Geron Corporation	No
hTERT oncolytic virus	Killing of telomerase positive cells	Cell Genesys (http://www.cellgenesys.com/)	No

TVAX, tumor vaccine; hTERT, human telomerase reverse transcriptase; hTR/hTERC, human telomerase RNA.

in cell proliferation [9]. The results of targeting hTERT mRNA via induction of anti-sense oligonucleotides are thus mixed.

Indirect inhibition of the reverse transcriptase component of hTERT by small nucleoside analogs has also been reported. Oligonucleotides, such as 6-thio-2'-deoxyguanosine 5'-triphosphate have been shown to be selective against telomeric DNA extension after its incorporation into DNA [10]. However, this inhibitor acts as a competitor for deoxyribonucleotide substrates that are required for the enzymatic function of hTERT [11].

The 11 base template telomerase RNA (hTR) is a target for direct enzymatic inhibition of telomerase activity. Exogenously added oligomers, such as 2'-O-MeRNA and peptide nucleic acid (PNA) inhibit telomerase leading to the shortening of telomeres and apoptosis after prolonged treatment [12].

Modified oligonucleotides with novel bond linkages have been reviewed by others [1,12,13]. These modified molecules bind RNA sequences with improved selectivity, enhanced efficacy and pharmacological properties analogous to DNA oligomers. Variations in the sugar phosphodiester backbone confer enhanced intracellular penetration, superior binding affinity, and specificity to the hTR RNA template to enable intact delivery into the target [13]. These forms of oligonucleotide hTR inhibitors require cationic lipid transfection for entrance into and efficacy within the cells.

A "telomerase template antagonist" agent (GRN163L) is about to enter into clinical trials [14]. The sequence 5'-Palm-TAGGGTTAGACAA-3' is complementary to a 13 nucleotide-long region partially overlapping and extending by four nucleotides beyond the 5'-boundary of the template region of hTR. This lipidated 13mer *thio*-phosphoramidate targets the hTR component of telomerase and does not require a lipid carrier because the lipid palmitate moiety is built into the molecule. GRN163L oligonucleotides form stable duplexes with single-stranded RNA, are negatively charged at neutral pH, are resistant to nuclease degradation, and display high specificity for DNA and RNA targets [15]. Duplex formation by the

oligonucleotide phosphoramidates are not substrates for RNase H hydrolysis *in vitro*, and have shown no overt toxicity at therapeutically effective biological doses.

Hammerhead ribozymes

Ribozymes are small sequence-specific catalytic RNA molecules capable of site-specific cleavage of target mRNAs. Introducing hammerhead ribozymes targeting hTR or hTERT mRNA into various cancer cells results in inhibited telomerase activity, telomere shortening and apoptosis [16,17]. Studies *in vivo* showed anti-tumor activity and decreased tumor size in mice treated with ribozymes targeting telomerase RNA [18]. However, one study showed that an hTR-targeting ribozyme decreased telomerase activity in melanoma cells but was unable to shorten telomeres and did not lead to complete growth inhibition [19]. Although xenograft animal studies have not been encouraging, the *in vitro* data show promise for this new group of telomerase inhibitors.

Reverse transcriptase inhibitors

Reverse transcriptase inhibitors are traditionally used for the treatment of HIV; nonetheless, these agents might also target the reverse transcriptase activity of telomerase for the treatment of cancer. The reverse transcriptase inhibitor 3'-azido-3'-deoxythymidine (AZT) inhibits telomerase activity; however, not all studies demonstrate progressive telomere shortening [20]. Oral squamous and mammary carcinoma cells show decreased telomerase activity and increased apoptosis following treatment with AZT. When used in combination, AZT enhances paclitaxel-induced cell apoptosis *in vitro* and augments the anti-tumor activity of paclitaxel in murine xenograft tumors, without host toxicity [21].

Dominant negative hTERT

Dominant negative hTERT (DN-hTERT) is a catalytically inactive form of hTERT that can sequester hTR. DN-hTERT effectively inhibits telomerase, causes progressive telomeric shortening, leading to apoptosis [22]. Subcutaneously

implanted leukemia cells expressing DN-hTERT do not form tumors compared to control vector or wild-type hTERT-expressing cells [23]. Combination treatment of DN-hTERT with various chemotherapeutic reagents, including cisplatin, docetaxel, and etoposide, enhances the sensitivity of lung cancer and melanoma cells to those agents; however, DN-hTERT increases the resistance of melanoma cells to temozolomide (TMZ) and carmustine [24,25]. Although there might be resistance owing to loss/methylation of the DN transgene expression or transcriptional up-regulation of endogenous hTERT [26], this remains an interesting gene therapy approach.

G-quadruplex stabilizers

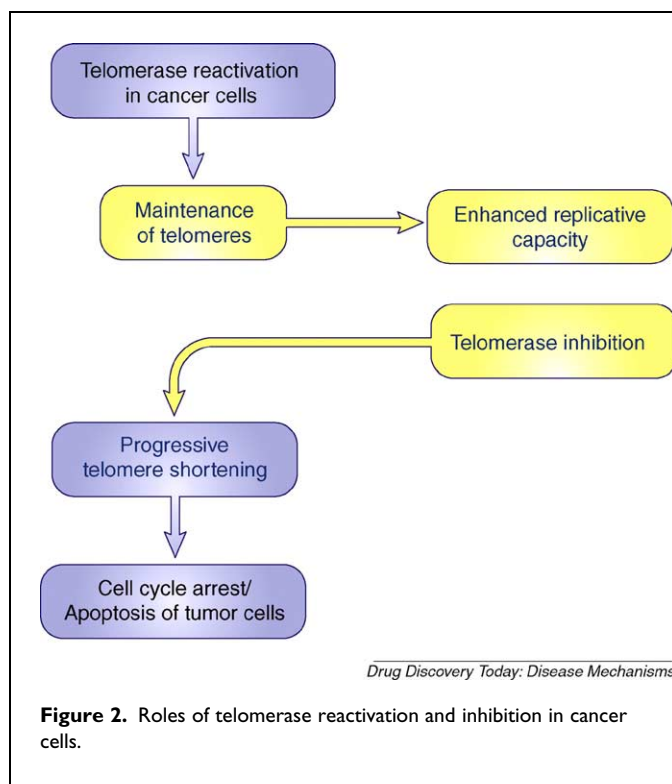
G-quadruplexes are structural four-stranded conformations formed by single-stranded telomeric DNA. Small molecules, such as anthraquinones, porphyrins, acridines and complex polycyclic systems, can inhibit telomere maintenance via stabilizing the quadruplex structure and inhibiting telomerase action [13]. Stabilization of G-quadruplexes in human uterus carcinoma cells resulted in the loss of nuclear hTERT expression, co-localization of hTERT with ubiquitin within the cytoplasm, growth inhibition of tumors in xenograft models [27], and telomere shortening and apoptosis in leukemia cells [28]. These studies show promise for future *in vivo* studies with G-quadruplex stabilizers.

Other approaches to inhibit telomerase

Abrogating Cdc42/Rac 1 (Gene ID 998) leads to decreased telomerase activity, demonstrating that signaling components can serve as targets of anti-telomerase therapy [29]. Perturbing proper assemblage of the telomerase holoenzyme with oligonucleotides that disrupt the P3/P1 pairing region or to the CR4–CR5 domain of hTR [30] prevents the proper assembly of telomerase leading to inhibited telomerase activity, but its effects on cell growth or apoptosis were not reported [30]. More recently, catecholic flavonoids, and natural products, such as rubromycins, alterperyleneol, diazaphilonic acid, epigallocatechin gallate and apigenin have been tested on telomerase [31]. These newly emerging strategies might prove useful if telomerase inhibition via these approaches can be proven to induce progressive telomere shortening and cell death.

Targeting telomere-independent functions of telomerase

In addition to the role of telomere maintenance (Fig. 2), telomerase can affect other areas including DNA-damage signaling, overcoming chemoresistance and regulation of apoptosis [8,21,32–36]. Surprising new data show rapid cell death following the inhibition of telomerase that might be independent of telomere length [8]. An alternative explanation is that there might be a population of short telomeres



in cancer cells that are immediately affected by telomerase inhibition leading to catastrophic telomere shortening. This might then activate a DNA damage signaling response that results in cell death, even when bulk telomeres are still long.

Some reports have shown no synergistic effects of combining telomerase inhibitors with chemotherapeutic agents, including paclitaxel, doxorubicin, etoposide, cisplatin or carboplatin, on the proliferation of cancer cells treated for a short-term (3 days) [33]. Other studies show that resistant tumor cells treated with telomerase inhibitors for only a short time become sensitive to other standard chemotherapeutic agents [21,34]; this effect occurs quickly, when the majority of telomeres have not yet eroded.

Overexpression of hTERT protects certain cancer cells from apoptosis via TRAIL signaling, but not from cell death due to etoposide or cisplatin [35]. In another study, apoptosis induced by telomerase inhibition was prevented by overexpression of Bcl-2 (Gene ID 596) and the caspase inhibitor zVAD-fmk, suggesting a site of action of telomerase before caspase activation and mitochondrial dysfunction [36]. The discovery of this novel anti-apoptotic role of telomerase, independent of telomere maintenance and its contribution to the rapid tumor cell death, warrants further investigation.

A new area is aimed at finding a clear explanation for the tumor-specific expression of hTERT [37]. Regulators of hTERT that are specific to cancer cells versus normal telomerase-positive cells might provide additional therapeutic targets.

Conclusions and perspectives

Although targeting cancer but not normal cells remains a potential issue, initial clinical trials in over 100 patients using telomerase immunotherapy have not shown evidence of adverse effects including bone marrow stem cells. Most peripheral blood T and B cells have decreased telomerase activity at rest, whereas there is an increase in telomerase expression in activated lymphocytes [38]. T cells stimulated with antigen show decreased expression of hTERT over time [39]; therefore, the side effects of telomerase inhibition on immune cells might also be limited. Immune cells are not easily infected by adenovirus, and thus the use of hTERT-promoter driven expression of oncolytic adenovirus might have few detrimental side effects on the immune system [1].

Outgrowth of telomerase-negative cells and resistance to telomerase inhibitors have been an important area of concern. With potentially new telomere-independent functions of telomerase being discovered, this problem might be minimized, because current data show rapid cancer cell death in the presence of a telomerase inhibitor, perhaps independent of telomere length. A combination of data has shown a sensitizing function of telomerase inhibitors making certain cancer cells less resistant to other standard chemotherapeutic agents. As ALT-like mechanisms are revealed, the importance of combination therapy will become increasingly more evident.

Challenges as well as triumphs exist in the preclinical and clinical testing of telomerase inhibitors as a therapeutic treatment option for cancer. Continued progress is being made in the study of telomerase biology and its role in cancer progression. As telomerase inhibitors move forward into human clinical trials, answers to its promising role should begin to emerge.

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