Telomerase Inhibitor: Promising Target Therapy for Cancer

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ABSTRACT

The key to selectively targeting cancer cells is to exploit some basic differences with their normal precursors. One such difference is the activity of the enzyme telomerase. Some studies targeted telomerase and telomeres in order to block limitless replicative potential of cancer cells, providing a fascinating strategy for a broad-spectrum cancer therapy. Telomerase based therapies could be well tolerated and perhaps with minimal side effect on telomerase-competent stem cells.

Keywords: Cancer therapy, molecular therapy, telomerase, telomeres

ABSTRAK

Kunci pengobatan selektif sel kanker adalah dengan memanfaatkan beberapa perbedaan mendasar antara prekursor sel kanker dan sel normal tubuh. Salah satu perbedaannya adalah aktivitas enzim telomerase. Beberapa penelitian sudah menargetkan telomerase dan telomer sebagai target terapeutik untuk memblokir potensi replikasi sel kanker yang tak terbatas, sebagai strategi terapi kanker spektrum luas. Terapi berbasis telomerase ini dapat ditoleransi dengan baik dan memiliki efek samping minimal pada telomerase sel normal tubuh lainnya. **Diana Kusuma, Izza Aliya, Andre Putra, and Mediana Sutopo. Penghambat Telomerase: Terapi Target Kanker yang Menjanjikan.**

Kata kunci: Pengobatan kanker, pengobatan molekuler, telomer, telomerase

The development of cancer treatment in the world today is fastly growing. A molecular approach has begun to take part for a higher rate of cancer-fighting success. The key to selectively targeting cancer cells is to exploit their some basic differences compared to normal precursors. One such difference is the activity of enzyme telomerase.[1,2]

Telomeres, Telomerase, and Cancer

Telomeres are G-rich non-coding tandem-repeated DNA sequences, located at the ends of eukaryotic linear chromosomes that preserve the coding region and maintain chromosomal integrity, bound by a series of single- and double-stranded DNA binding proteins termed the *shelterin* complex.[3] When the telomere reaches a critical length, it triggers the DNA damage response machinery and leads to cellular senescence or apoptosis.[4]

Telomeres will be 50–200 bp shorter 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. In cells that do not express telomerase, progressive shortening of the telomere occurs with each cell division that ultimately results in cellular (replicative) senescence. Conversely, cancer cells have active telomerase that maintain telomere length, providing those cells with unlimited cell division. Telomere length is also typically shorter in tumor cells compared to adjacent noncancerous cells replicative senescence.[3]

Telomerase, which consists of hTERT (telomerase reverse transcriptase catalytic subunit) and hTR components (a functional telomerase RNA (template)), is responsible for the maintenance of telomeres. The introduction of the catalytic subunit of telomerase (hTERT) into telomerase silent cells is often sufficient to produce telomerase activity, elongate or maintain telomeres, and to result in the bypass of both senescence and critical phase. This demonstrates that other components of telomerase holoenzyme are present in normal telomerase silent cells and only the expression of the hTERT protein is absent in most normal cells. Thus, telomeres appear to count the number of times a cell has divided, and determine when cellular senescence occurs.Telomerase is expressed in almost all cancer cells but is inactive in most normal somatic cells.[5] By contrast, approximately 85% of immortal cancer cell lines express high level of telomerase and maintain a short telomere length. Therefore, the presence of telomerase is critical for cell immortalization and tumorigenesis.[4] (**Figure 1**).

Anti-telomerase therapies are likely to be most effective when used after tumorreduction surgery and in combination with other standard therapies such as chemotherapyand radiotherapy. In addition, the combination of novel targeted therapeutics,such as angiogenic inhibitors together with telomerase inhibitors, areespecially attractive

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and are currently being tested in at least one ongoing clinical trial.[3] In this instance, the angiogenic inhibitors would keep the tumor size small but dividing, while telomerase inhibitors would gradually lead totelomere shortening with each cell division resulting in the induction of apoptosis and potentially durable responses.

Telomerase inhibitors may also be effective in reducing the risk of relapse by targeting the small numbers of telomerase-positive cancer cells in adjacent tissues not removed during tumor resection, while telomerase is down-regulated in non dividing cells, even if there is a subset of more quiescent cancer cells,eventually they also would have to proliferate and thus in the presence of a telomerase inhibitor even these cells should eventually display progressive telomere shortening.[13]

TELOMERASE INHIBITORS

1. Inhibiting hTERT

a. Immunotherapy

In cancer cells, the degradation of telomerase by proteasomes results in the formation of protein fragments or peptides of telomerase that are expressed on the tumor cell surface as antigens by the human leukocyte antigen (HLA) class I pathway, and these telomerase antigenic epitopes can be targeted by cytotoxic T cells to destroy the tumor cells. Telomerase-specific epitopes can induce CD4+ or CD8+ cytotoxic T-lymphocyte responses or stimulate antigen-presenting cells capable of attacking tumors. Therefore, the rationale for anti-telomerase immunotherapy is to sensitize the immune system to tumor cells expressing hTERT peptides to activate and generate hTERT-specific CD8+ cells to produce enhanced anti-tumor effects. Two major strategies have been adopted to develop effective telomerase-based immunotherapy in cancer: an hTERT vaccine approach and a dendritic cell approach to prime antigen-presenting cells ex vivo. Vaccines targeting telomerase Human hTERT-specific epitopes are expressed on cancer cells but not on normal cells. Telomerase (hTERT) is thus regarded as a universal tumor antigen owing to its expression in almost all cancers.[5] Three hTERT vaccines, GV1001, Vx001 and GRNVAC1, have been used to elicit anti-telomerase immune responses in cancer patients.[6]

b. Gene Therapy

There are two general approaches to telomerase gene therapy; suicide gene therapy and oncolytic viral therapy.

- Suicide 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 is driven expression of TRAIL. The Ad-hTR-NTR adenoviral delivery of the suicide gene therapy construct consists of the activation of the nitroreductase enzyme (NTR) by the hTERC (telomerase functional RNA) promoter and the addition of a prodrug. Telomerase regulation of a suicide gene therapy approach is the novel component of this therapeutic plan.[3,8]

- Oncolytic virus gene therapy

This approach utilizes adenoviruses that have been manipulated or engineered to have oncolytic, or cancer-killing, properties, enabling them to selectively target and destroy cancer cells that express telomerase. The promoter region of the telomerase (hTERT) gene regulating the replication of adenovirus permits selective viral propagation within cancer cells but not normal cells that do not express telomerase. Thus, telomerase-expressing tumor cells containing the telomerase-specific virus eventually rupture and die theoretically spreading the virus to adjacent cells. When these same engineered viruses infect normal somatic cells, there is no replication or killing effects. This approach is known as a Tumor-specific Replication competent ADdenoviral (hTERTp-TRAD) gene therapy.[3,8]

c. Small-Molecule Telomerase Inhibitors

Most researchers aim to seek natural agents or synthesize chemical compounds to inhibit or abolish the telomerase activity in cancer cells and destruct the telomere maintenance

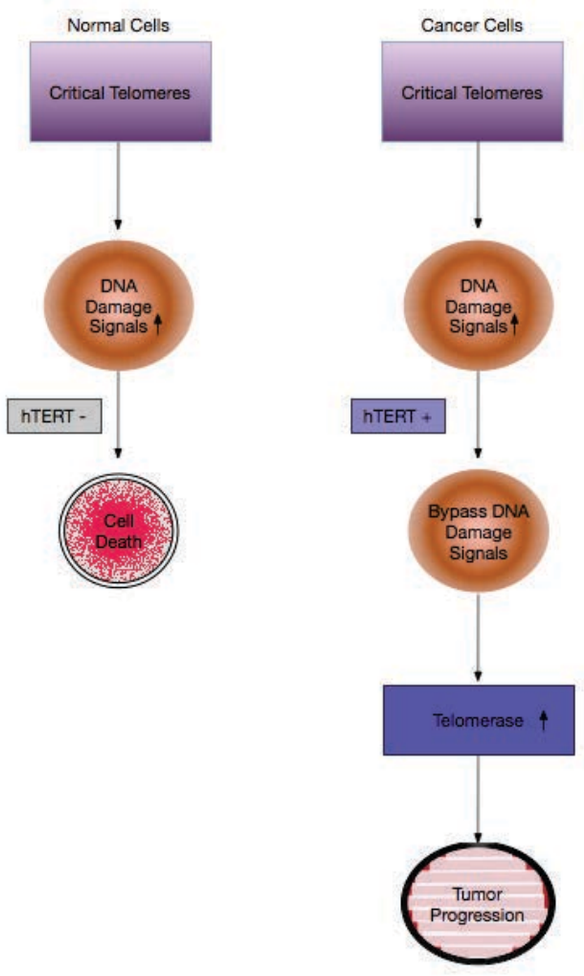

Figure 1. Normal cells vs cancer cells.

mechanism, leading the cancer cells to growth senescence and apoptosis. Most telomerase inhibitors are aimed directly at the core component of telomerase: the TERT andthe RNA component TERC.

BIBR1532{2-[(E)-3-naphtalen-2-yl-but-2-enoylamino]-benzoic acid}, small non nucleotidic synthetic compound, is a non-competitive inhibitor which is distinct from nucleosidic compounds or antisense oligonucleotides. It is proposed that BIBR1532 does not block the template copying steps but impairs translocation of the enzyme DNA substrate complex or may promote the enzyme dissociation from the DNA substrate during catalyzing. BIBR1532 targets directly the telomerase core components hTR and hTERT and induces the cancer cells to growth arrest with telomere shortening and dysfunction.

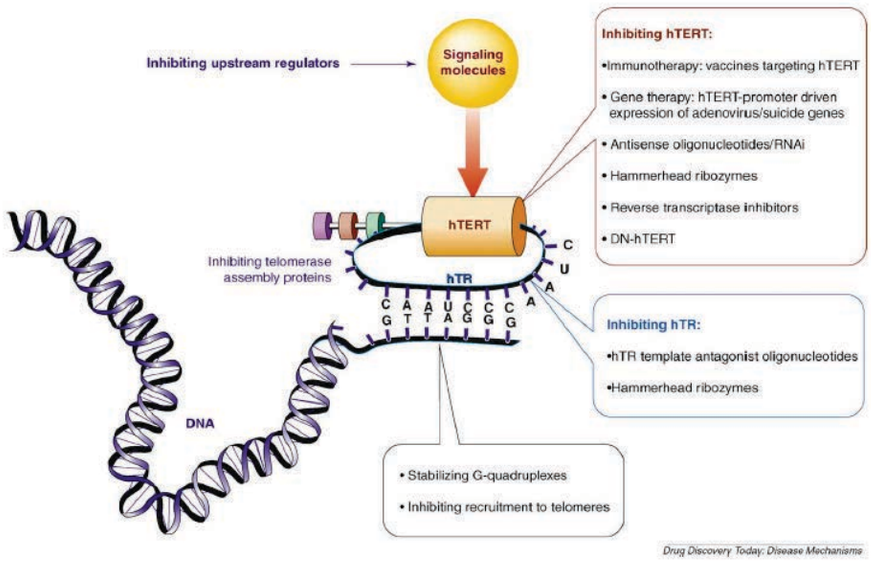

Figure 2. Multiple targets of telomerase inhibition.[5]

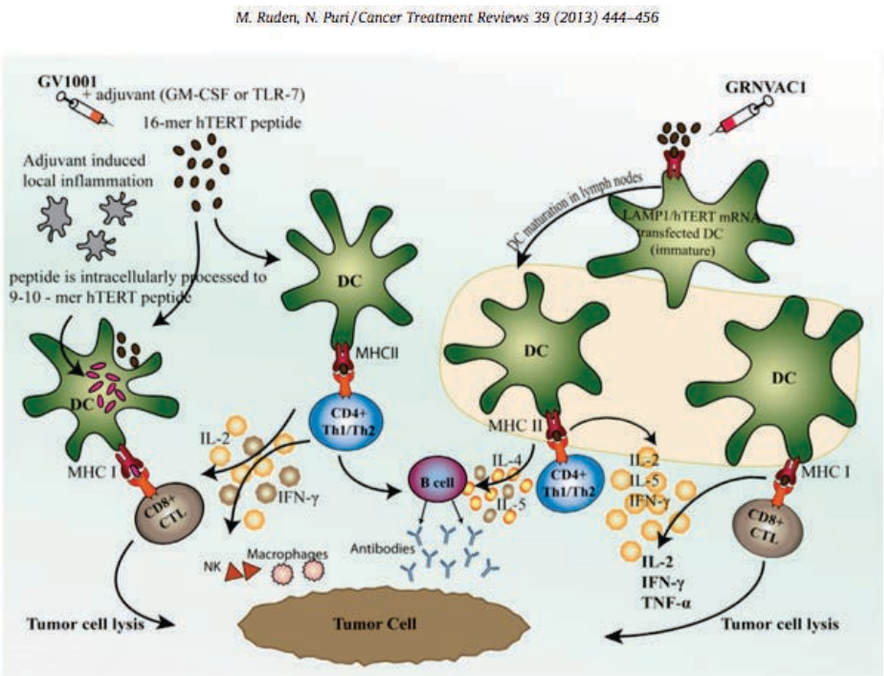

Figure 3. The immunotherapy approach as a successful method.[7]

TNQX (2, 3, 7-trichloro-5-nitroquinoxaline) is a mixed-type non-competitive inhibitor, which can suppress the telomerase activity both significantly and selectively. MCF7 cell line treated long term with a non-cytotoxic concentration of the TNQX resulted in progressive telomere erosion followed by chromosome abnormalities and senescence. The mechanism of telomerase inhibition by the TNQX is inducing chromosomal end-to-end fusion, thus leading to incomplete DNA replication and cell arrest in late S phase.

Pyrimethamine which induces apoptosis and matrix metalloproteinases (MMP) andpotent telomerase inhibition in the prostate cell line is considered to be a chemopreventative agent in cancer. Lately, it has been found that the "T-oligo", an oligonucleotide which has the homology sequence to the terminal telomeric DNA, is a telomerase inhibitor and has shown cytotoxic effects in multiple cancers and destruction of the T-loop structure followed by the DNA damage response likely pathway.[9]

d. Hammerhead Ribozymes

Ribozymes are RNA molecules which possess specific endoribonuclease activity and catalyze the hydrolysis of specific phosphodiester bonds, resulting in the cleavage of the RNA target sequences. After the cleavage reaction, the substrate is accessible by ribonucleases, a step that guarantees its permanent inactivation and offers a considerable advantage over the simple physical blockage obtained with complementary oligomers. The ability of a hammerhead ribozyme, directed against the RNA component of human telomerase, to inhibit the catalytic activity of the enzyme.[10]

e. Reverse Transcriptase Inhibitor

The reverse transcriptase inhibitor 30-azido-30-deozythymidine (AZT) inhibits telomerase activity; however, not all studies demonstrate progressive telomere shortening. 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.[9,11]

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 telomericshortening, leading to apoptosis. Subcutaneouslyimplanted leukemia cells expressing DN-hTERT do not formtumors compared to control vector or wild-type Htertexpressing cells. Combination treatment of DN-hTERTwith various chemotherapeutic reagents, including cisplatin,docetacel, and etoposide, enhances the sensitivity of lungcancer and melanoma cells to those agents; however, DNhTERT increases the resistance of melanoma cells to temozolomide (TMZ) and carmustine.[5]

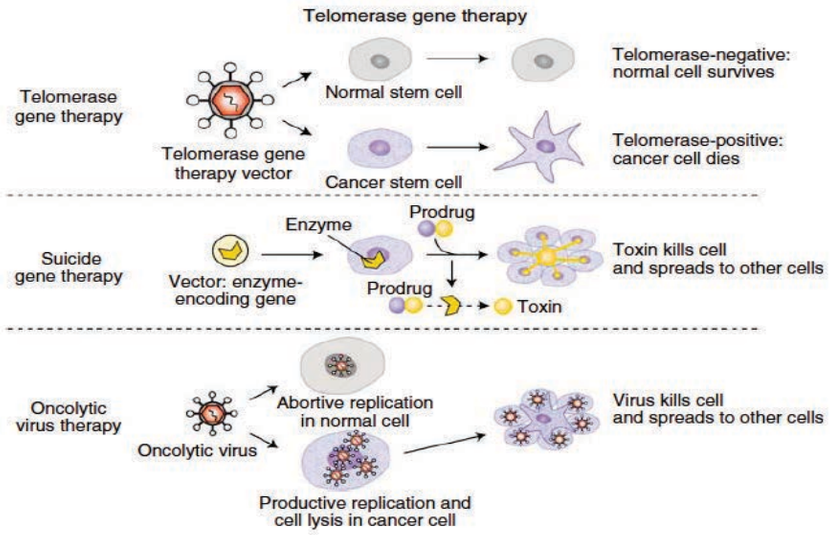

Figure 4. Telomerase gene therapy approaches.[8]

2. Inhibiting hTR (hTR Template Antagonist)

The telomerase complex offers several opportunities for targeting, such as genetic manipulation of expression, template antagonism or direct enzymatic inhibition. One such target is the telomerase RNA component, hTR, which is required for the RNA-dependent reverse transcriptase function of hTERT. The steps involved in synthesizing telomeres by telomerase include: The binding of the short C-rich template sequence region of hTR to the G-rich telomeric strand by Watson Crick base pairing; elongation of the telomere by hTERT, in which six nucleotides (GGTTAG) are added sequentially, driven by the template sequence of hTR; and translocation of the telomere-template heteroduplex to position it for another round of elongation (processive addition).

This process can be disrupted when the extended telomere is dissociated from telomerase. The hTR is not translated into peptides; therefore, oligonucleotides complementary to the hTR template region block the active site of hTERT reverse transcriptase and act as competitive enzyme inhibitors (or template antagonists).[12]

3. G- Quadruplex G-quadruplex-Interacting Ligands

Telomere G-overhang could form G-quadruplex structures in vivo. Ligands that selectively bind to G-quadruplex, which stabilizes this structure, may interfere with telomere conformation and elongation. The common features of these ligands include a large flat aromatic surface, presence of cationic charges, and the ability to adopt a terminal stacking mode. Derivatives of acridines, cationic porphyrins, triazines, anthraquinones, and perylenes, such as BRACO-19,TMPyP4, RHPS4, and telomestatin, can promote and stabilize the G-quadruplex structure, leading to disassociation of the telomere-associated proteins and cell apoptosis. BRACO-19, 3, 6, 9-trisubstituted acridine compound, decreased hTERT expression drastically with the cellular senescence and complete cessation of growth in the human uterus carcinoma cell line UXF1138L. In vivo, BRACO-19 was highly active as a single agent against early-stage tumors and with corresponding loss of nuclear hTERT protein expression and an increase in atypical mitoses indicative of telomere dysfunction. BRACO-19 is predicted to interact selectively with the human DNA quadruplex structure thus inhibiting the capping and catalytic functions of telomerase.[9]

CONCLUSION

Most human cancers have short telomeres and have increasing telomerase enzyme activity. These aspects are responsible to infinity of cancer's growth and cancer cells life cycle. Telomerase is an attractive target for cancer diagnosis and therapy. Targeting telomerase as a therapeutic approach for cancer would be expected to lead telomere's shortening and further could induce cancer cell death with minimal side effects on normal cells. Telomerase based therapies could be well tolerated and perhaps with minimal side effect on telomerase competent stem cells.[3]

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