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REVIEW

Telomerase activity: An attractive target for cancer therapeutics

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Abstract

Telomeres are non-coding tandem repeats of 1000-2000 TTAGGG nucleotide DNA sequences on the 3′ termini of human chromosomes where they serve as protective "caps" from degradation and loss of genes. The "cap" at the end of chromosome required to protect its integrity is a 150-200 nucleotide-long single stranded G-rich 3′ overhang that forms two higher order structures, a T-loop with Sheltering complex, or a G-quadruplex complex. Telomerase is a human ribonucleoprotein reverse transcriptase that continually added single stranded TTAGGG DNA sequences onto the single strand 3′ of telomere in the 5′ to 3′ direction. Telomerase activity is detected in male germ line cells, proliferative cells of renewal tissues, some adult pluripotent stem cells, embryonic cells, but in most somatic cells is not detected. Re-expression or up-regulation of telomerase in tumours cells is considered as a critical step in cell tumorigenesis and telomerase is widely considered as a tumour marker and a target for anticancer drugs. Different approaches have been used in anticancer therapeutics targeting telomerase. Telomerase inhibitors can block directly Human TElomerase Reverse Transcriptase (hTERT) or Human TElomerase RNA telomerase subunits activity, or G-quadruplex and Sheltering complex components, shortening telomeres and inhibiting cell proliferation. Telomerase can become an immune target and GV1001, Vx-001, I540 are the most widespread vaccines used with encouraging results. Another method is to use hTERT promoter to drive suicide gene expression or to control a lytic virus replication. Recently telomerase activity was used to activate pro-drugs such as Acycloguanosyl 5′-thymidyltriphosphate, a synthetic ACV-derived molecule when it is activated by telomerase it does not require any virus or host active immune response to induce suicide gene therapy. Advantage of all these therapies is that target only neoplastic cells without any effects in normal cells, avoiding toxicity and adverse effects of the current chemotherapy. However, as not all the approaches are equally efficient, further studies will be necessary.

Key words: Human telomerase reverse transcriptase; Immunotherapy; Suicide gene therapy; Acycloguanosyl 5′-thymidyltriphosphate; Telomerase inhibition

Core tip: One of the hallmark of cancer is the replicative immortality of tumor cells guaranteed by telomerase activity that counteracts progressive telomere shortening during cellular replication: this makes telomerase a tumor marker and a target for anticancer drugs. In this review we summarize and update the most recent innovative studies and results on the different strategies that consider telomerase as a target for cancer therapy. In particular, we try to point out the advantages and the potentialities of some innovative approaches, compared to other, equally promising, but that need further investigations.

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TELOMERES, TELOMERASE AND CANCER

Telomeres are non-coding tandem repeats of 1000-2000 TTAGGG nucleotide DNA sequences on the 3' termini of human chromosomes[1-3] where they serve as protective "caps" from degradation and loss of genes. In this way cells can discriminate between double strand breaks and natural chromosome ends[4,5]. In human somatic cells, telomeres become critically short after successive cell divisions (number of divisions depending on the length of their telomeres), cells stop division and replicative senescence occurs[6]. As a consequence, telomeres can reach a critical length that is no longer suitable to assemble into T-loop: this triggers a localized DNA damage response and p53-mediated cell cycle arrest[7-9]. However, cells that have inactivated the p53-pathway cell cycle checkpoint, are able to continue dividing, bypassing senescence, loosing telomeric sequence with each division[9,10] and reach a "crisis" stage[11,12]. In this way telomeres become so short that cannot protect chromosome ends, so that they fuse together to produce a dicentric chromosome, inducing an increase aneuploidy and genomic instability that finally will lead to p53-independent apoptosis[13,14]. Bypassing crisis rarely occurs in human cells (1 in 10^{-6} in epithelial cells and 1 in 10^{-7} in human fibroblasts) and this leads to cell immortality and cancer cell progression, characterized by capability to continue to proliferate without limits.

The "cap" at the end of chromosome required to protect its integrity is a 150-200 nucleotide-long single stranded G-rich 3' overhang that forms two higher order structures, a T-loop with Sheltering complex, or a G-quadruplex complex. Sheltering complex is represented by six proteins (TRF1 and TRF2, POT1, TPP1, TIN2, RAP1) responsible for maintaining the T-loop structure. G-quadruplex is stabilized with BRACO19, RHS4 and telomestatin proteins. Sheltering complex with T-loop, G-quadruplex and its stabilizers can lock the telomeric 3' overhang and block telomerase from accessing telomeres[15] (Figure 1).

Telomerase is a human ribonucleoprotein reverse transcriptase that continually adds single stranded TTAGGG DNA sequences onto the single strand 3' of telomere in the 5' to 3' direction and translocates to the new terminus[16,17]. This cycle goes on as far as telomerase dissociates from telomere[18,19]. Telomerase is composed of two main subunits: the catalytic protein Human TElomerase Reverse Transcriptase (hTERT) and the ribonucleoprotein template Human TElomerase RNA (hTER)[15-17]. In particular hTER consists of 451 nucleotides of which only nucleotides 46 through 56 (5'-CUAACCCUAAC-3') represent a template for new telomeric added DNA sequences (Figure 2).

Many proteins associated to the core components hTERT and hTER are required and are necessary for stability regulation, recruitment and activity of the holoenzime[20]. hTER is expressed in all human cells, as well as normal and tumour cells, so telomerase activity is limited by of hTERT expression, whereas is present[21,22].

Telomerase activity is detected in proliferative cells of renewal tissues, in some adult pluripotent stem cells, male germ line cells, embryonic cells, but not in most somatic cells[23]. However, telomerase activity is found in almost all human cancer cell lines and in about 85%-90% of primary tumours[24]. In fact, one of the hallmark of cancer is the replicative immortality and so the ability to endlessly growth is synonymous of telomerase reverse transcriptase reactivation. Up-regulation or re-expression of telomerase in tumour cells is considered as a critical step in cell tumorigenesis and telomerase is widely considered as a tumour marker and a target for anticancer drugs. Progressive telomere shortening during cellular replication is counteracted by telomerase activity[1,25].

One of the advantages of anticancer therapies targeting telomerase is that the telomeres of highly proliferating cancer cells are shorter (5 kb) compared to that in normal somatic cells and stem cells (10-20 kb) that have not yet reached critical lengths as a result of aging[26,27]. The difference in telomerase activity and telomere lengths in normal and cancer cells leads to a more selective therapeutics cytotoxicity on cancer cells and a minimal impact on normal cells with a limitation of collateral effects that can be evaluated[28].

TELOMERASE INHIBITION AS A THERAPY

Telomerase inhibitors can be employed as a selective anticancer therapy, disrupting telomerase-positive cancer cells replicative capacity[29].

To target telomerase in cancer treatment we can find two types of approaches: the first one is blocking directly telomerase hTERT or hTER subunits activity, with consequent shortening of telomeres leading to the arrest of cell replication. The second approach is to block telomerase by an indirect method, targeting G-quadruplex stabilizers or Sheltering complex components with the consequence of preventing telomerase interaction with telomeres or binding of proteins associated with telomerase; this leads to telomere uncapping and cell apoptosis[30].

Antisense olignucleotides-targeting hTER

One of the most recent strategy for a direct telomerase enzymatic inhibition, is the use of antisense oligonucleotides inhibitors. These molecules are complementary to the 11-base template region of telomerase (hTER) and can be used to block the translation of sense RNA. In order to hybridize the hTER-template the antisense oligonucleotides must get to the hTER region without being degraded by nucleases. For this reason the challenge for this kind of drugs is both access and stability. To better get its target, antisense oligonucleotides have been modified and significantly improved in the past years.

Currently GRN163L (Imetelstat®) is one of the first generation most promising telomerase inhibitor targeting hTER used in cancer treatment; it is a lipid modified

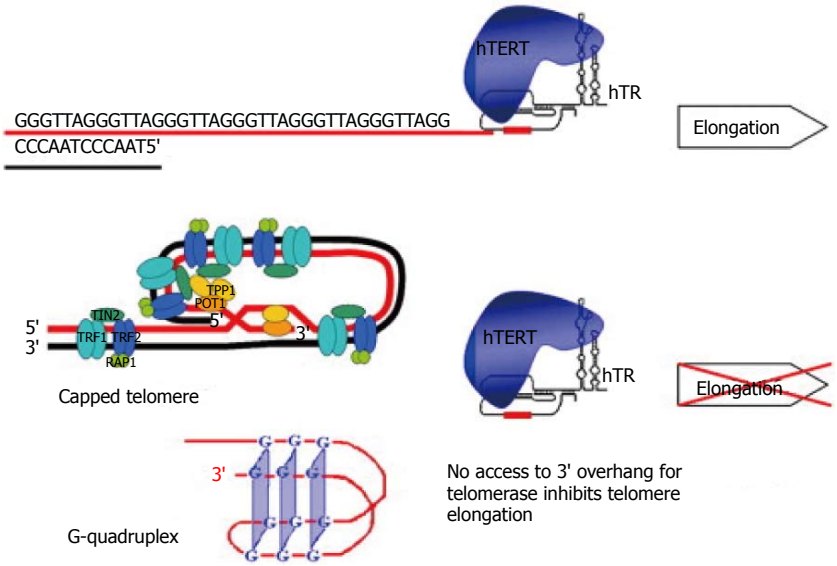

Figure 1 Impact of open or capped telomere structure on the telomerase activity. (Reproduced with permission from Philippi C, Loretz B, Schaefer UF, Lehr CM. J Control Release 2010; 146: 228-240. Copyright Clearance Center, Inc.). hTERT: Human TElomerase Reverse Transcriptase.

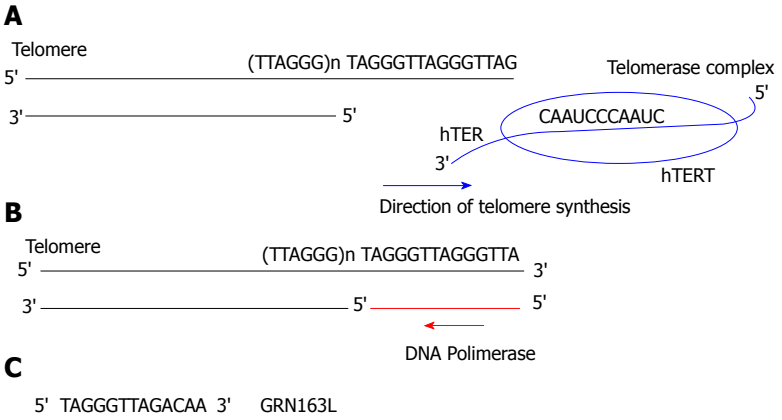

Figure 2 Telomeres an telomerase complex. (TTAGG)n sequences form a 3'-overhang on the 3' end of chromosome. Telomerase is composed by hTERT and hTER subunits; hTER is the RNA template for DNA synthesis to add new telomeric TTAGG sequences on 3'-overhang; B: DNA polymerase completes the lagging strand; C: GRN163L sequence complementary. hTERT: Human TElomerase Reverse Transcriptase; hTER: Human TElomerase RNA.

version of GRN163, a 13-mer oligonucleotide N3'-P5'-thio-phosphoramidate, that required a lipid carrier molecule and a lipid-base transfection agent to adequately enter tissue and cellular membranes[31,32]. On the contrary, GRN163L with a covalently bound lipophylic palmitoyl (C16) group linked to its 5'-thio-phosphate[33] is lipid soluble, and shows an higher drug availability and bio-distribution, without any lipid carrier supply[32]. GRN163L in part overlaps the hTER template region by binding with high affinity and specificity at its active site, acting as competitive telomerase inhibitor and causing a total enzyme inhibition[32,33] (Figure 2).

The GRN163L inhibitory effect on telomerase activity has been evaluated in different cancer cell lines[34] and its effects were evident as well as "*in vitro*" and "*in vivo*" models; in fact, long term treatment with GRN163L reduced cell viability in cancer cells derived from bladder[33] glioblastoma[35], multiple myeloma[36], Barrett's adenocarcinoma[37], as well as breast[38,39], lung[40], liver cancer[41] and prostate[42].

Recently, the effects of GRN163L have been tested on a panel of ten pancreatic cancer cell lines, and the results indicated that the inhibitory effect of the drug was maintained also after its removal[43]: in fact, only three weeks after the GRN163L removal, a telomerase recovery was observed, but the enzyme was less processive. This suggests that to maintain continuous telomerase inhibition and to reduce side effects risk after a pharmacological treatment of a patient with GRN163L, a maintenance dose given once every other week might be sufficient. However, the reversible effects of Imetelstat have been also previously demonstrated on rat mesenchymal stem cells[44].

A combined treatment where homologous recombination and telomerase inhibition are associated, causes a significant increase in telomeres attrition, relative to each treatment alone, leading to senescence and apoptosis in Barrett's adenocarcinoma[45].

Tamakawa *et al*[46] showed that the DNA damage induced in S/G_2 phase of the cell cycle, by genotoxic stimulus was potentiated by the telomerase inhibition induced by GRN163L in breast and colorectal cancer cells[46].

In previous studies, synergies between GRN163L and various anticancer treatments such as microtubule inhibition, inhibition of oncogenic signals and ionizing radiation, were considered to be dependent on longer-term changes associated with chromatin status[47] and telomere length[48].

Telomere shortening induced by telomerase inhibitors would affect the self-renewal properties of cancer stem cells (CSCs), normally not responding to standard chemotherapy, but capable of inducing initiation and currency

in different hematologic and solid tumours[49,50].

Many studies showed that CSCs can represent the Imetelstat target in different cancers[35,42,51], and that a telomere shortening-independent as well as dependent Imetelstat mechanism of action on CSCs subpopulation, can be suggested[52,53]. The effect of Imetelstat was evaluated on both the bulk cancer cells and putative CSCs of breast and pancreatic cancer cell lines. The *in vitro* treatment inhibited telomerase activity, cell growth, self renewal in bulk cancer cells and putative CSCs, with a consequent reduced cancer engraftment in nude mice[52]; in particular an increased sensitivity of CSCs to Imetelstat did not correlate with differences between telomerase activity expression levels or telomere length of CSCs and bulk tumour cells suggesting a telomere shortening- independent mechanism of action for the Imetelstat effects on CSCs subpopulation.

All these studies support the hypothesis that conventional therapies often fail to target CSCs while the use of telomerase inhibitor could have the potential role for more durable clinical response in many tumors, reducing relapse recurrence.

Imetelstat is currently in phase Ⅱ clinical development for breast cancer, non-small cell lung carcinoma, multiple myeloma, and other tumor types[30].

Inhibitors targeting hTERT: BIBR1532

BIBR1532 [2-(E-3-naphtalen-2-yl-but-2-enylylamino]-benzoic acid] is actually a promising hTERT inhibitor among the few TERT inhibitors developed. BIBR1532 is a small synthetic non-nucleic compound that linking hTERT in its active site, inhibits telomerase in a non-competitive manner: BIBR1532 does not cause chain termination events but rather leads to an overall reduction in the number of added TTAGGG repeats[54]; in particular the drug could act translocating the enzyme-DNA-substrate complex, or favouring the DNA substrate disjunction from the enzyme during the copy of the template[55].

In the last few years, different studies showed that BIBR1532 treatment induced telomerase activity reduction with consequent cell growth arrest in different human cancer cell lines[54,56-60], without affecting normal stem cells[61]. In addition telomeres targeting might represent a valid strategy for the re-sensitization of chemoresistant chondrosarcomas[56], and a rapid induction of a high level telomere dysfunction appears to be a crucial parameter for the development of future telomerase-based therapeutic[62]. However, although some human squamous cell carcinoma cell lines are resistant to telomerase inhibition[63] some works suggest that a valid strategy for the treatment of both drug-resistant and drug-sensitive cancers may be pharmacological telomerase inhibition in combination therapy[64-66].

IMMUNOTHERAPY FOR TELOMERASE EXPRESSING CANCER

As previously described, nearly all cancer cells over-express functional active telomerase, and hTERT-specific epitopes are expressed on tumour cells, but not on normal cells. In this way, telomerase become an immune target, and can be eradicated by the stimulation of the immune system with specific vaccines. Telomerase-target immunotherapy sensitizes immune cells against tumor cells expressing hTERT peptides as surface antigens[67]. The consequent expansion of telomerase-specific CD8+ cytotoxic T lymphocytes is directed to target and kill telomerase positive cancer cells[68,69].

Recently, multiple peptides are known to induce hTERT-specific immune responses[68] and several vaccine strategies are being developed and used: among these GV1001, Vx-001, I540, are the most widespread therapeutic approaches. As almost all human tumor-associated antigens are self-proteins, their specific T cells are often tolerated: this is the major problem of cancer immunotherapy. For this reason, overcoming tumor-specific self-tolerance is a principal goal in cancer immunotherapy.

Self-tolerance is commonly directed against "dominant" (high affinity for HLA) but not against "cryptic" (low affinity for HLA) peptides[70,71], so the simplest way to circumvent tolerance is to use these cryptic peptides[72] as for example Vx-001 (9-mer cryptic TERT 572 peptide) that was developed as tumour-associated antigen of hTERT to induce cytotoxic T lymphocyte responses[73,74].

Immunological response associated with extended survival were evident in patients with advanced non-small-cell lung cancer treated with Vx-001 vaccine (TERT572Y peptide)[74]; in patients with various types of chemo-resistant advanced solid tumours (stages Ⅲ and Ⅳ) the vaccination with Vx-001 stimulates TERT572-specific reactive T cells in a great number of patients independently of the disease stage or clinical status before vaccination and a late immune response correlated with longer survival was induced[73,75].

State of the art of clinical trials using anti-telomerase cancer immunotherapy is encouraging. In fact, vaccines are tested in breast, lung, melanoma, prostate, and pancreatic cancer[76-82] and these trials have widely induced a specific immune response against hTERT positive cancer cells. Encouraging results have been also obtained in patients with advanced melanoma, where immunity to hTERT has been safely generated[83]. The combination of cancer vaccination with chemotherapy showed that temozolomide and GV1001 induced immune and clinical response in 78% of stage Ⅳ melanoma patients, that developed long-term T-cell memory and survived more than those rapidly losing their responses[84]. Vaccination with GV1001 was well tolerated and immunized the great part of non-small cell lung cancer patients establishing durable T-cell memory[85]. However, GV1001 vaccination was not effective in cutaneous T cell lymphoma patients, raising concerns about also its safety[86]. The survival data indicated that patients with non-resectable pancreatic cancer treated with GV1001 showed that immune response correlated with an extended survival, suggesting that the vaccine could be the new goal for pancreatic cancer patients treatment and encouraging further clinical

studies[82]. On the contrary, in patients with advanced and metastatic pancreatic cancer the use of GV1001 telomerase vaccination in combination with chemotherapy, induced a weak and transient immune response and did not improve overall survival[80,81]. Likewise, a low dose cyclophosphamide treatment in combination with GV1001 vaccination in patients with advanced hepatocellular carcinoma did not show antitumor efficacy[87]. Further studies and new strategies are needed to analyze and to enhance the immune response effect of telomerase vaccination during chemotherapy, in patients with both pancreatic and hepatocellular cancers.

Vaccination with autologous dendritic cells transfected with hTERT mRNA (GRNVAC1) represents another anticancer approach that induced immunological response in human. Immunotherapy targeting the hTERT subunit of telomerase has been demonstrated to induce an important immune responses in cancer patients after vaccination with single hTERT peptides, while vaccination with dendritic cells transfected with hTERT mRNA has a key role in inducing efficient immune responses to multiple hTERT epitopes. In this way this kind of therapy can be an attractive approach to more efficient immunotherapy[88-90].

TELOMERASE-EXPRESSING CELLS AS TARGET OF ONCOLYTIC VIRUSES

Recently has been shown that the use of hTERT promoter to drive the expression of a suicide gene and/or control the replication of a lytic virus, can be a successful approach to target cancer cells.

To drive the expression of a suicide gene, the expression of a pro-apoptotic protein, like TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) or pro-drug-activating enzyme[91-96] is controlled by the hTERT promoter, generally active in cancer cells expressing telomerase. These cells are injected with viruses carrying the suicide gene and then killed by a toxin derived from the administration of a pro-drug activated by the pro-drug-activating enzyme.

A second clinical approach, is to use the hTERT promoter to control the replication of a lytic virus. Oncolytic effects on tumors can be mediated by oncolytic viruses, tumor selective viruses genetically modified and engineered to replicate in and kill only cancer cells. For this purpose, the *E1* gene expresses viral proteins E1A and E1B necessary for adenovirus replication, but the modified virus can replicate only in cells which express telomerase if gene itself is redesigned to be controlled by the hTERT promoter[97-100]. One such virus is telomelysin (OBP-301) that in pre-clinical studies targets selectively only telomerase-expressing cells.

The modified viruses induce cytolysis in several kinds of human cancer cell lines in which can replicate; when human lung, prostate or liver cancer cells were used in xenotransplantation models, intratumoral injection of the virus reduced tumor growth and improved mice survival[97-100].

The potential role of oncolytic virotherapy has recently been demonstrated to be a promising strategy in the management of human gastrointestinal cancer[101]. Studies about OBP-301 have been shown that it mediates the effective *in vivo* purging of metastatic tumor cells from regional lymph nodes and moreover it co-operates to optimize treatment of human gastrointestinal malignancies[102]. Moreover, telomerase-specific oncolytic viruses is a potential treatment of human squamous cell carcinoma of head and neck[103], while in pancreatic cancer the combination therapy with gemcitabine has been tried, exhibiting enhanced cytotoxic effects both "*in vitro*" and "*in vivo*"[104]. In addition, preclinical study showed that OBP-301 can be used for treatment of human hepatocellular carcinoma and that its tumor-killing activity persists after multiple injections[105].

Data regarding combination therapy with OBP-301 and chemotherapeutic agents are preliminary but encouraging[106]. In particular Boozari *et al*[107] showed that the combination of intratumoural virotherapy with an antitumoural vaccine, could represent a promising immunotherapeutic strategy against hepatocellular carcinoma and metastasis.

TELOMERASE CANONICAL ACTIVITY AS A THERAPY

Recent studies revealed that telomerase canonical activity can be exploited for therapeutic purpose.

The evidence that telomerase is expressed in almost all tumor cells, preventing telomeres shortening by continually adding single stranded TTAGGG DNA sequences, prompted us to develop a thymidine analogue pro-drug, acycloguanosyl 5'-thymidyltriphosphate (ACV-TP-T) (Figure 3). This molecule is a synthetic ACV modification that is metabolized by telomerase, and this reaction releases the active form of acyclovir able to reduce pancreatic and hepatocellular carcinoma cells growth as well as "*in vitro*" and "*in vivo*"[108,109].

ACV is a nucleoside analogue acting as a DNA chain terminator that could be used in the suicide gene therapy[110]. ACV or the ACV analogue ganciclovir[110,111] when used as antiviral agent needs a first phosphorylation to ACV monophosphate by herpes virus thymidine kinase (TK) carried by wild-type herpes virus or, in the suicide gene therapy, by engineered adenovirus (Figure 3), then cellular kinases perform the two remaining phosphorylation to obtain the ACV triphosphate. This active metabolite is incorporated into DNA during its replication causing DNA chain termination.

On the contrary, ACV-TP-T, may be metabolized by telomerase that incorporates thymidine in replicating telomeres and releases ACV diphosphate. This process skips the viral TK phosphorylation, allowing the cellular kinases to go on with further phosphorylation to obtain the active drug[108,109]. The results showed that after activation of ACV-TP-T by telomerase, cell proliferation is significantly

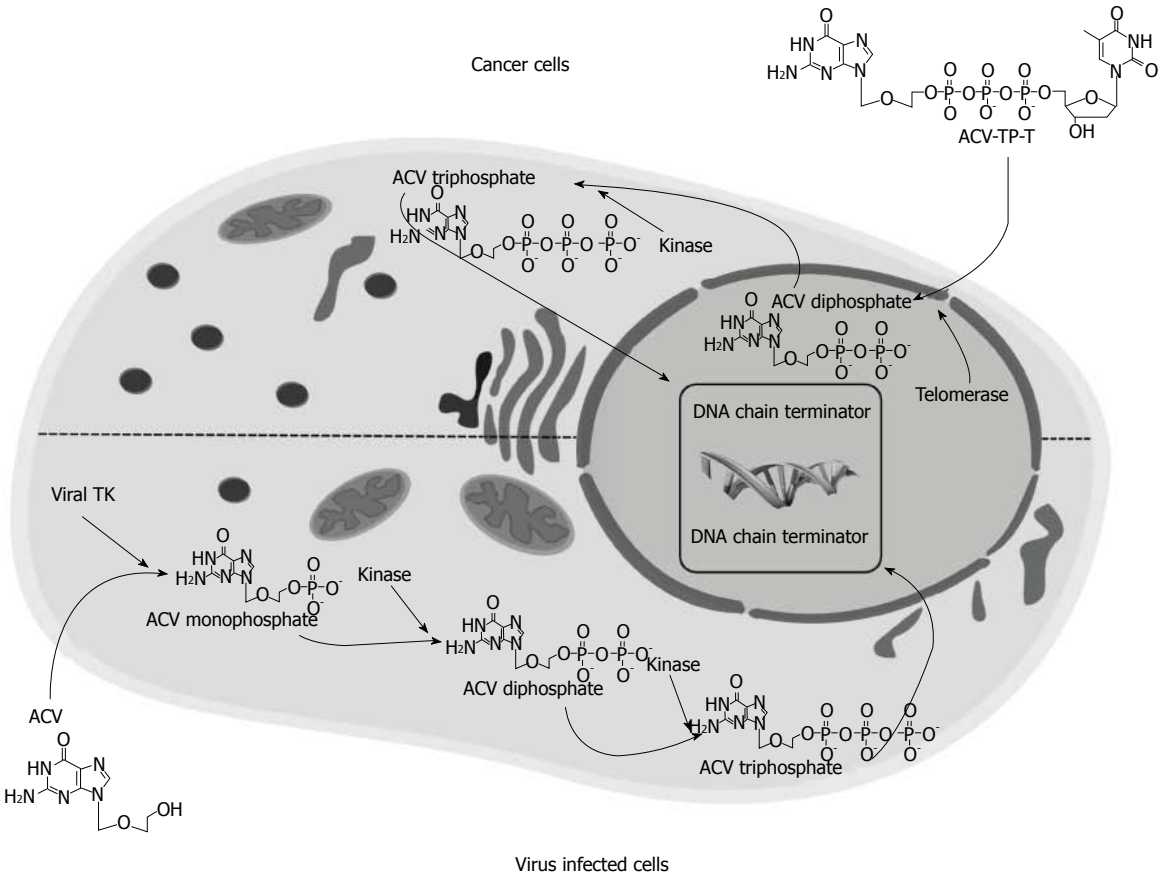

Figure 3 Structure and schematic mode of action of Acycloguanosyl 5'-thymidyltriphosphate in comparison with ACV. For activation, ACV requires to be phosphorylated to ACV monophosphate by viral TK carried either by wildtype herpes virus or, in the suicide gene therapy, engineered adenovirus. ACV monophosphate is then further phosphorylated by cellular kinases to the triphosphated active form. Conversely, ACV-TP-T is substrate of telomerase that incorporates the thymidine in the replicating telomeres and directly release ACV diphosphate skipping the viral TK phosphorylation step. (Reproduced with permission from Ref [108]. Copyright 2011 AGA Institute). ACV-TP-T: Acycloguanosyl 5'-thymidyltriphosphate; TK: Thymidine kinase.

reduced and apoptosis is increased in different human pancreatic adenocarcinoma cell lines. High and low telomerase activity is related with low and high IC_{50} of the drug, respectively. On the other hand, the cytosine-containing pro-drug ACV-TP-dC, which is not a telomerase substrate, is not able to reduce pancreatic cancer cell proliferation. Moreover, ACV-TP-T administration increases apoptosis, reduces growth, proliferation and vascularization of pancreatic xenograft tumors in mice[108].

Analogue results were obtained in human and murine hepatocellular carcinoma cell lines and in transgenic and orthotopic murine models of hepatic cancers[109]. Furthermore, in orthotopic syngenic mice, ACV-TP-T has been used alone or in combination with the approved standard of care, Sorafenib, a multikinase inhibitor. Combination therapy showed a synergistic effect between Sorafenib and ACV-TP-T.

Advantages of this strategy are evident. Despite recent improvements in suicide gene therapy, the application of adenovirus-mediated therapy is limited by many factors: the low and transient expression levels of the transgene[110,112,113], the induction of immune response in the host[108], and a late carcinogenesis[112]. In addition ethical concerns regarding the use of virus in patients[112,113] could be a limitation.

The use of telomerase promoter[114] and the introduction of conditionally replication-competent adenovirus[115] only partially overcome the above mentioned disadvantages. Moreover, the immunotherapy based on vaccination for telomerase[84] relies on the induction of an active immune response that often is deregulated in the oncology treated patients[116].

In this contest, the use of ACV-TP-T represents a new therapeutic strategy that exploits the enzymatic activity of telomerase. This approach is efficient only in neoplastic cells without any effects in normal cells, it avoids the toxicity and the adverse effects of the current chemotherapy, and finally, it does not require the use of any viruses or an active immune response of the host.

As a paradox in this contest telomerase switches from being a target of anticancer therapy, to an integral part of the therapy. Preliminary evidences suggest the possible use of ACV-TP-T molecule for the treatment of other tumors characterized by high telomerase expression and activity such as ovarian and adrenocortical cancers.

NON CANONICAL EFFECTS OF TELOMERASE

Telomerase activation may have both telomere-dependent

and telomere-independent implications for cancer progression: in particular, telomerase reverse transcriptase may exert some biological functions independently of its telomere maintenance enzymatic activity.

Different studies support a role of telomerase in some telomere-independent activities in cancer progression; nevertheless, apart from its role in telomere maintenance, the molecular mechanism by which telomerase promotes cancer is still not fully understood. Zhou *et al*[117] showed that hTER regulated vascular endothelial growth factor (VEGF) expression at the transcriptional level, independently of telomerase activity[117]; previous studies reported that VEGF induced hTERT expression and activity in normal[118] and cancer cells[119]. All these results suggested a positive feedback regulation that could contribute to a mutual and collaborative function of VEGF and telomerase in cancer progression.

Wu *et al*[120] in a recent review focused on various signaling pathways and genes involved in the feedback regulation of TERT. The expression of numerous genes involved in different cellular processes, as well as cell cycle and cellular signaling, could be regulated by TERT, indicating that telomerase is both an effector and a regulator in carcinoma. However, the mechanisms underlying the interaction between TERT and its target genes are still not completely understood.

Ghosh *et al*[121] suggested a functional interplay between TERT and nuclear factor (NF-κB) signaling, further reinforced by the observation that telomerase over expression resulted in enhanced expression of NF-κB target genes, whereas telomerase null mice were refractory to NF-κB activation; in addition, it seems that also hTER could regulate the expression of some NF-κB target genes. The function of hTER in gene expression regulation is not clear, in fact, hTERT can form complexes with or without hTER[122].

hTERT could be involved also in a negative feedback loop system with pRb/E2F pathway in cancer, as well as in a positive feedback loop with Wnt/β-catenin signalling, or in multiple interactions with phosphoinositide 3 kinase/Akt pathway[120]. In addition, Liu *et al*[123] demonstrated a potential role of hTERT in epithelial mesenchymal transition.

Although the mechanisms underlying the interaction between TERT and its target genes are still not completely understood, all the above observations, strengthen the idea that telomerase non-telomeric functions could be used as a new therapeutic target for cancer.

CONCLUSION

Although recent and ongoing results support an important role for telomerase targeting therapeutics in cancer treatment, additional preclinical and clinical trials are necessary to improve some of these strategies.

In fact, if difficulties with dendritic cells derivation will be easily overcome[124], vaccination with dendritic cells transfected with hTERT mRNA could potentially become an attractive approach to a more potent immunotherapy. In addition, further studies are necessary to enhance the effects of telomerase vaccination in combination with intratumoral virotherapy and with standard chemotherapeutic agents.

On the contrary, beside more promising approach offered by GRN163L that seems to target also CSC, BIBR1532 could be preferred therapy if used also in combination with standard chemotherapy for the treatment of drug-resistant cancers.

Finally, ACV-TP-T use is very promising and deserves further studies. In fact, preclinical evidences showed that this new pro-drug may be considered for treatment of hepatocellular and pancreatic carcinoma, as well as of other tumors characterized by high telomerase expression and activity.

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