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Review

Therapeutic Targeting of Telomerase

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Abstract: Telomere length and cell function can be preserved by the human reverse transcriptase telomerase (hTERT), which synthesizes the new telomeric DNA from a RNA template, but is normally restricted to cells needing a high proliferative capacity, such as stem cells. Consequently, telomerase-based therapies to elongate short telomeres are developed, some of which have successfully reached the stage I in clinical trials. Telomerase is also permissive for tumorigenesis and 90% of all malignant tumors use telomerase to obtain immortality. Thus, reversal of telomerase upregulation in tumor cells is a potential strategy to treat cancer. Natural and small-molecule telomerase inhibitors, immunotherapeutic approaches, oligonucleotide inhibitors, and telomerase-directed gene therapy are useful treatment strategies. Telomerase is more widely expressed than any other tumor marker. The low expression in normal tissues, together with the longer telomeres in normal stem cells versus cancer cells, provides some degree of specificity with low risk of toxicity. However, long term telomerase inhibition may elicit negative effects in highly-proliferative cells which need telomerase for survival, and it may interfere with telomere-independent physiological functions. Moreover, only a few hTERT molecules are required to overcome senescence in cancer cells, and telomerase inhibition requires proliferating cells over a sufficient number of population doublings to induce tumor suppressive senescence. These limitations may explain the moderate success rates in many clinical studies. Despite extensive studies, only one vaccine and one telomerase antagonist are routinely used in clinical work. For complete eradication of all subpopulations of cancer cells a simultaneous targeting of several mechanisms will likely be needed. Possible technical improvements have been proposed including the development of more specific inhibitors, methods to increase the efficacy of vaccination methods, and personalized approaches. Telomerase activation and cell rejuvenation is successfully used in regenerative medicine for tissue engineering and reconstructive surgery. However, there are also a number of pitfalls in the treatment with telomerase activating procedures for the whole organism and for longer periods of time. Extended cell lifespan may accumulate rare genetic and epigenetic aberrations that can contribute to malignant transformation. Therefore, novel vector systems have been developed for a 'mild' integration of telomerase into the host genome and loss of the vector in rapidly-proliferating cells. It is currently unclear if this technique can also be used in human beings to treat chronic diseases, such as atherosclerosis.

Keywords: telomerase; telomeres; aging; senescence; atherosclerosis; cancer; gene therapy; immunotherapy; regenerative medicine; personalized medicine

1. Telomeres and Telomerase in Aging and Cancer

Aging is a complex process, which is accompanied by cycle arrest, remodeling in cell morphology and chromatin structure, functional decline, and extensive shifts in gene expression and metabolism. The aging of human cells can be mediated via stress-related mechanisms and via replicative senescence

induced by telomere shortening. The various senescence triggers interact cooperatively and induce overlapping signaling pathways. Stressors include endogenous substances, exogenous factors, and species-specific mechanisms of aging such as replicative senescence. Replicative aging induced by telomere attrition is a species-specific aging mechanism, which acts as a tumor-suppressor in large, long-lived organisms [1] (Figure 1). Telomere attrition is, however, associated with functional decline and other negative effects that become relevant for the organism beyond the stone-age life span of approximately 50 years. An inverse correlation between telomere length and onset of age-related diseases has been shown in many studies even though the causality is still controversial [2].

Telomeres consist of repetitive non-coding DNA sequences (in humans TTAGGG), which are located at the end of the chromosomes. Telomeres, together with the shelterin complex, form a cap to protect the chromosome ends [3–5]. The shelterin complex consists of six telomere-associated proteins [6]. The telomere sequence is recognized by the subunits TRF1, TRF2, and POT1. These subunits are interconnected by the proteins TIN2, TPP1, and Rap1. The complex allows cells to distinguish telomeres from DNA damage sites. Without this protection, e.g., when telomeres shorten beyond a critical threshold, unprotected telomeres provoke a DNA damage response [7].

Telomere shortening occurs due to the so-called end replication problem, which means that the 3′ end of the DNA strand shortens with each cell division, since the DNA polymerase cannot completely replicate the strand [5,8]. At a certain threshold of telomere attrition the damage-repair system recognizes the unprotected DNA double strand as DNA breaks and activates the p53 or the p16INK4a signaling pathway to initiate a senescence or apoptosis program. Reactive oxygen species (ROS) or other environmental stress factors may also lead to telomere damage and accelerate the telomere attrition. Particularly, the GGG triplet within the human telomere sequence TTAGGG is vulnerable to chemical modifications. From a critical telomere length, onwards, telomeres are unable to claim the shelterin complex resulting in loss of the protective inner nucleotide loop, which ultimately leads to genomic instability [9,10] (Figure 1).

In numerous studies, it was observed that a healthy lifestyle is correlated with longer telomeres, likely reflecting protection against age-related diseases [4]. It has been shown in aging mice that cells with short and/or damaged telomeres are accumulating in stress-prone tissues, likely due to replicative exhaustion and/or stress-induced telomere damage. Animal studies suggest that senescence is not only a marker of, but also involved in, the propagation of age-related disorders [5,10].

From an evolutionary point of view, it is thought that the cell division limit was developed as a mechanism for tumor suppression. Indeed, in mice short telomeres are a hindrance to cancer growth. On the other hand, very short and damaged telomeres can also provoke tumor growth when a missing cap leads to chromosomal instability, as it is exemplarily observed in Dyskertosis congenita [11].

Telomere length and cell function can be preserved by the reserve transcriptase telomerase. The human telomerase consists of two subunits: a RNA templates (TERC, telomerase RNA component), and the catalytic subunit (hTERT, human telomerase reverse transcriptase), which synthesizes the new telomeric DNA from the RNA template [4]. Higher telomerase activities are under normal conditions only detectable in cells that need a high replicative capacity, such as stem cells and progenitor cells [9].

Telomere elongation by telomerase leads to chromosomal stabilization and a change to a more youthful gene expression pattern. In addition to its established role in extending telomeres, hTERT can promote proliferation of resting stem cells through a non-canonical pathway [9] and has direct effects on transcription and cell signaling, e.g., as a cofactor in a β-catenin transcriptional complex [12], which plays a role in embryogenesis and development [13]. Uncontrolled induction of telomerase would, however, have also pitfalls. Telomerase, per se, is no oncogene, but permissive for carcinogenesis and approximately 90% of all tumor cells express the enzyme to elongate the telomeres, which makes its use for systemic applications problematic.

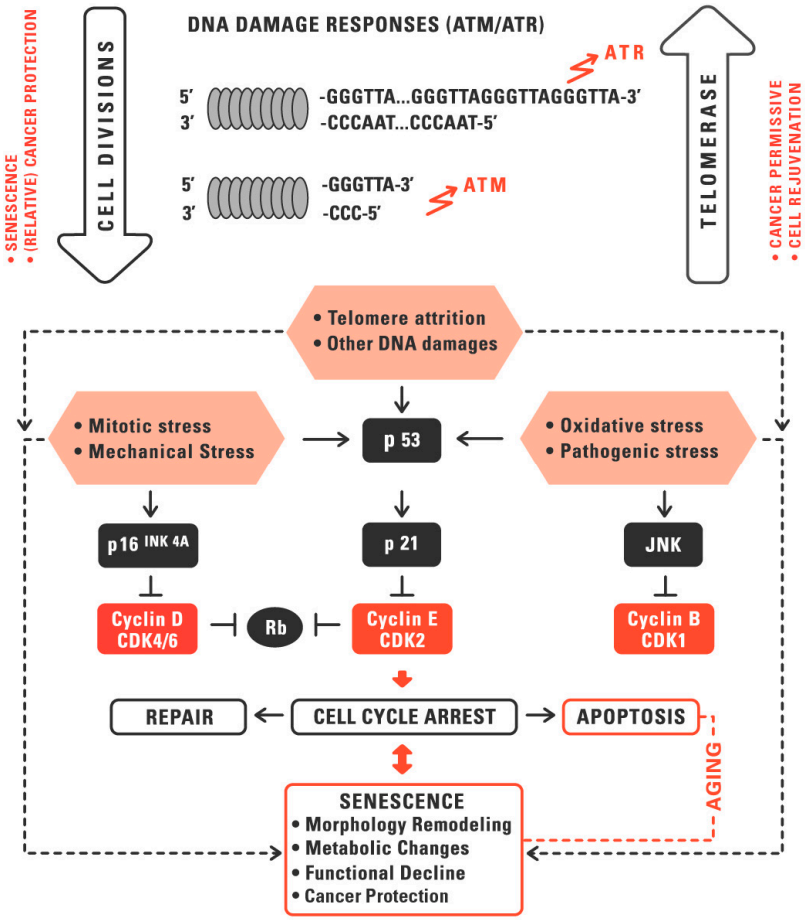

Figure 1. Replicative aging cooperates with other aging mechanisms to activate the p53 and/or Rb signaling pathways. ATM and ATR are sensors of DNA double- and single-strand damage induced by replicative senescence or other DNA damage. Activated ATM and ATR trigger checkpoint responses to induce cell cycle arrest. A stronger stimulation of p53 may lead to apoptosis by activating the mitochondrial pathway of apoptosis. Telomere length and cell function can be preserved by the reserve transcriptase telomerase, which synthesizes the new telomeric DNA from the RNA template. Telomerase may help to avoid senescence and to rejuvenate tissues, but is also permissive for carcinogenesis. Senescence may help to prevent tumor growth but can also be overcome by a process called crisis, and then has paracrine and other pro-tumorigenic effects. With permission, adapted from [1].

The variety in telomere length in individuals of the same age is determined by genetic and environmental factors, leading to telomere damage and accelerated shortening of telomere length [14,15]. Inflammation is thought to contribute to telomere attrition in cells of the immune system by promoting leukocyte turnover and replicative exhaustion, and possibly also by direct modulation of telomerase activity by ROS and other stress factors [16]. For example, increased production of cytokines has been shown to adversely affect telomerase activity and telomere length [17]. C-reactive protein (CRP), a marker of inflammation, is inversely correlated with leukocyte telomere length (LTL) [18]. Shorter telomeres are associated with higher interleukin-6 (IL-6) and C-reactive protein values [19]. Telomerase activity was found to be reduced by psychological and life stress [20,21]. Various stressors trigger increased ROS formation, which leads to telomere attrition both directly and indirectly (by lower hTERT activity), which ultimately leads to a reduction of leukocyte telomere length (LTL) [4]. Smoking is an excellent example for higher ROS formation and, consequently, progressive shortening of telomeres [22].

At the cellular level, senescence serves as a natural tumor suppressor [23]. Senescent cells are no longer capable of replication and shut down their metabolism to a minimum. Only some key pathways are active and only few genes are expressed at higher levels. The senescence normally prevents the replication of abnormal chromosomes. The p16/pRb tumor suppressor pathways are activated in response to DNA damage and telomere dysfunction during senescence [23–25]. This process, however, could be flawed by oncogene activation to bypass senescence. An incorrect removal of senescent cells can lead to malignancy [23]. The alternative lengthening of telomeres (ALT) mechanism enables cancer cells with inactive telomerase the conservation of the telomere structure [26,27]. Approximately 5%–10% of cancer cells maintain their telomeres by ALT, in which sister chromatids exchange their telomeres by non-reciprocal recombination events [28]. Studies have shown that these cancer cells are more sensitive to ROS and drug treatments when they elongate their telomeres by ALT. Apparently these cells are under strong pressure to activate the alternative mechanism to escape senescence and apoptosis [26].

The catalytic subunit hTERT (human telomerase reverse transcriptase) was found to be upregulated in cervical carcinomas [29,30], hepatocellular carcinoma [31]], lung tumors [29], breast carcinomas [29], and neuroblastomas [29]. Telomerase in tumor cells is re-expressed on transcriptional, post-transcriptional, post-translational, and epigenetic levels [23]. Under normal conditions, the absence of CAAT and TATA elements in the TERT promoter prevents constitutive activation. However, promoter mutations or unusual epigenetic changes may overcome this barrier [32]. Telomerase also plays a regulatory role in the spread of cancer cells [33]. In the vast majority of investigated tumors an increased expression of human telomerase RNA (hTR) was detected, as well [34].

Telomerase is a target for both anti-cancer and cell rejuvenation strategies with a broad overlap of targets at different cellular and functional levels (Figures 1 and 2).

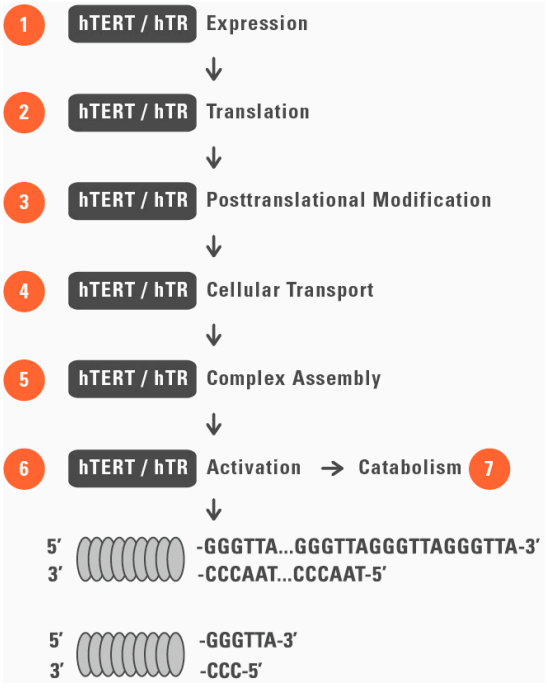

Figure 2. Therapeutic targeting of telomerase. Potential targets of telomerase for antitumor (telomerase suppressing) and rejuvenation (telomerase activation) drugs are shown by numbers 1–7. 1, inhibition/activation of gene transcription; 2, inhibition/activation of protein synthesis; 3, modulation of activity by posttranslational modifications; 4, modulation of telomerase activity by cellular sequestration; 5, interference with telomerase complex assembly; 6, modulation of signaling pathways and molecules involved in enzyme activation, such as Wnt/β-catenin, PI3K/Akt, and mTOR signaling; and 7, modulation of telomerase complex catabolism including vaccine therapy.

2. Telomerase as a Target for Regenerative Medicine

Replicative senescence contributes to the decline in many physiological functions and in most tissues and, thus, contributes to the pathology of chronic diseases [35,36]. As telomerase activity is not, or only at low levels, detectable in somatic tissues there are many situations and chronic diseases in which the transient rejuvenation by telomerase immortalization could be a therapeutic option [16,37,38]. There are several possible strategies to reconstruct or enhance the enzymatic activity for therapeutic use:

(1). Classical gene therapy with transfection of telomerase sequences: This approach can be used for tissue engineering, for in vitro optimization of stem cell transplantation in donor cells with short telomeres [39] and, in principle, also for the treatment of chronic diseases in the whole organism, provided that induction of telomerase is time-limited.

(2). Re-expression of silenced telomerase: Cell differentiation normally leads to transcriptional downregulation of telomerase induced by signaling and epigenetic alterations [40,41]. However, telomerase downregulation can, at least in part, be reversed by various substances and mechanisms. Examples are histone deacetylase inhibitors [42] and estrogen receptor agonists, the latter acting by Akt mediated phosphorylation [43]. Many drugs with main targets other than telomerase also influence hTERT on transcriptional and/or posttranslational level. Involved signaling pathways that upregulate hTERT expression and/or activity (see also paragraphs below) are PI3/Akt, MAPK/ERK1/2, and the Wnt/β-catenin pathway.

(3). Activation of residual enzymatic activity: Activation of telomerase activity itself is an option for cells with residual telomerase activity such as stem cells of regenerative tissues and lymphocytes. In lymphocytes' clonal expansion typically activates telomerase activity via enzyme phosphorylation and subsequent nuclear translocation [44]. This function declines with advanced age and leads to exhaustion of memory cells and could be restored by direct interaction with the telomerase holoenzyme or the telomerase activating signaling pathways [45].

(4). Modulation of the intracellular location: The sequestration of telomerase is another possible level of regulation on telomerase activity, implicating telomerase localization as a potential target for pharmacotherapy [46]. Telomerase can be translocated between the nucleus and the cytosol. hTERT is also present in mitochondria with yet unknown physiological significance [16,47].

Ectopic expression of telomerase was used to immortalize a wide variety of cell types including human fibroblasts [48–52], dermal fibroblasts [53,54], keratinocytes [55], muscle cells [56–58], vascular endothelial [59–61], myometrial [62], retinal [48–52], bone marrow stromal cells [63–66], osteoblasts [67–69], odontoblasts [70], CD4 and CD8 T cells [71,72], mesenchymal stem cells [72], myoblasts [73], hepatic stellate cells [74,75], fetal neuronal precursors [76], and breast epithelial cells [37]. Some cell types, such as bronchial and corneal cells, were used to form three-dimensional cultures [37].

Telomerase reconstruction was first discussed for treatment of diseases with distorted enzymatic activity of telomerase, namely, dyskeratosis congenital and aplastic anaemia [77]. Potential other applications are production of epithelia for burns or wounds, endothelia for blood vessels, chondrocytes for the treatment of arthritis, osteocytes for bone defects, and hematopoietic cells for bone marrow transplants or for the replacement of immune cells [39,78,79]. By use of this technique human blood vessels have already been engineered in vitro [56].

Transient telomerase activation may also be used for the treatment of other chronic diseases such as cardiac muscle disease, atherosclerosis [15], immunodeficiency, and bone marrow failure [11,80], liver disease [11,81], pulmonary fibrosis [11,82], degenerative cartilage defects [83], cataract [84], rheumatoid arthritis [85], organ transplantation [86], or treatments associated with the accelerated formation of senescent cells such as past cancer therapy or HIV [87,88]. Cartilage defects have become the target of cartilage tissue engineering [83]. Thomas and coworkers have demonstrated that bovine

TERT-modified bovine adrenocortical cells can be transplanted into severe combined immunodeficient mice, and that these cell clones behave like their normal counterparts and form functional tissue after transplantation. This tissue is histologically similar to tissue formed from normal cells and shows a similar rate of cell division, implying a therapeutic role of telomerase in xenotransplantation [86].

The association between telomere length and aging has also led to the development of telomerase activators that may induce hTERT and/or hTR expression, enhance enzyme activity and/or influence cellular location. The idea behind this approach is to reverse normal cellular aging and to treat symptoms of aging. A single molecule telomerase activator, cycloastragenol (commercially available as TA-65, derived from *Astragalus membranaceus* root), has been shown to transiently activate telomerase in T lymphocytes [87], associated with the retardation of telomere shortening, increased proliferative potential, and enhanced functional response [88]. This substance was proposed to be used for the treatment of accelerated immunosenescence in HIV patients to increase the number of senescent memory CD8 T cells [87,88]. Cycloastragenol (TA-65) has been sold as a food supplement since 2013 and has been identified as an effective telomerase activator in immune cells, neonatal keratinocytes, and fibroblasts [87,89], acting via ERK-pathway activation and subsequent enhancement of telomerase expression. It increases the telomere length in mice without increasing the cancer incidence [90]. In a small pilot study it was used for the treatment of age-related macular degeneration [91] and was shown to improve markers of metabolic, bone, and cardiovascular health [92]. A moderate increase in leukocyte telomere length was shown in humans [93]. However, the number of patients in these studies was limited and some effects were of borderline significance. Long-term prospective studies regarding positive or side-effects are lacking.

Other phytochemicals have been shown to activate telomerase. Resveratrol activates telomerase in mammary epithelial [94] and endothelial progenitor cells [95], most likely due to the upregulation of SIRT1 [96]. Current knowledge regarding possible long-term effects is, also for this substance, incomplete [96]. For the treatment of cataracts pharmaceuticals with telomerase activating effects such as N-acetylcarnosine have been proposed since reduced telomere length is intimately involved in opacification, making the lens opaque or cloudy [84]. Another compound (AGS-499) has neuroprotective effects in mice and showed delayed progression of amyotrophic lateral sclerosis and increased survival in SOD1 transgenic mice [97]. Bone marrow mesenchymal stem cells often display premature aging und unstable proliferation. It has recently been shown in a rat model that co-transfection of BMSCs with telomerase and nerve growth factor had a better effect on learning and memory compared to cells lacking these factors [98]. These effects may be used for the development of therapeutic strategies to treat cognitive impairment in vascular dementia.

Indirect strategies to upregulate telomerase activity are described for certain antioxidants, such as N-acetylcysteine, which blocks the nuclear export of telomerase into the cytosol [99] and α-tocopherol, which was shown to retain telomerase activity in brain microvascular endothelial cells [100]. The idea behind this concept is that ROS damage telomeres directly (by damaging the vulnerable GGG triplet of the repetitive telomere sequence) and indirectly (by modulating telomerase activity and cellular location) [101]. HMG-CoA reductase inhibitors may also have telomere lengthening effects [102], by interfering with the redox balance of cells [99] and by increasing expression of the telomere stabilizing protein TRF2 [103]. Finally, Ginkgo biloba was shown to activate telomerase by inducing PI3K/Akt signaling [104].

Telomerase upregulation in skin diseases: The skin is an organ for which some therapeutic approaches of telomerase are already in use. Several therapeutic strategies have been proposed based on in vivo or ex vivo stimulation of stem/progenitor cells for expressing hTERT or telomere RNA component (hTR) and for the replacement of dysfunctional or lost skin [37,105–111]. The application area ranges from reconstructive surgery after severe wounds, burns, deep skin injuries, infections, and decubitus ulcers [106,107] to treatment of diseases with defects in hTERT and/or hTR associated with premature replicative senescence of the skin, e.g., the premature aging Werner syndrome, in Fanconi anaemia, and chronic dysplastic anaemia [37,112]. Positive effects of bone marrow-derived stem/progenitor cells for

skin tissue engineering have been shown in several studies either alone or in combination with artificial skin grafts, thereby reducing the risk of graft rejection [106,108,113]. Ex vivo co-culture of human skin substitutes with circulating endothelial progenitor cells improved survival by formation of functional microvessels [107]. In a rat model transfection of hTERT into hair follicle stem cells by coating polyethylenimine DNA complexes on the skin surface stimulated hair growth [114], presumably by both telomere elongation and/or modulating Wnt/β-catenin signaling. Recently, optimized three-dimensional culture conditions have been described with enhanced hTERT expression levels, proliferation, and multipotency of human dermal stem/progenitor cells [115].

Telomerase upregulation in atherosclerosis: Despite enormous research and identification of numerous risk factors are the exact causes of atherosclerosis incompletely understood and the exact pathomechanism remains unclear [4,116]. Experimental findings in cell culture and animals suggest that telomere shortening contribute to the pathogenesis of atherosclerosis at advanced age. Numerous findings in humans have shown that telomere shortening correlates with the degree of atherosclerosis in vivo [15]. Experimental data suggest that the activation of telomerase can delay and—at least in part—reverse the senescent phenotype [15,117,118]. The pathologically vicious circle between replicative aging and inflammation by atherosclerosis could be reversed by a telomerase-based therapy [119]. Matsushita et al. have shown that a stable hTERT expression in endothelial cells ensures a younger phenotype and induces improvement of endothelial nitric oxide synthase (eNOS) [120]. Telomerase and vascular endothelial growth (VEGF)-mediated angiogenesis potentially regulate the transcriptional expression of each other, suggesting a role of telomerase in regulating cellular processes other than telomere elongation, such as differentiation and angiogenesis [119]. Development of therapeutic approaches is still on an experimental level due to fear of cancer-promoting side-effects on systemic use.

Telomerase upregulation in psychiatric disorders: Based on experimental and preliminary clinical data it was hypothesized that the mode of action of many psychopharmacological drugs (e.g., antidepressants, lithium, and antipsychotics) is, at least in part, mediated by their influence on telomerase activity. A close correlation between stress-dependent telomerase activity and depression-like behaviors has been shown in mice [121]. Fluoxetine reversed clinical symptoms and increased hippocampal telomerase activity in parallel, raising the possibility that drug effects might be mediated by telomerase-dependent neurogenesis [121]. Smaller pilot studies suggested a close correlation between telomerase activity, clinical symptoms and response to antidepressants [122,123]. Likewise, lithium increased hippocampal telomerase activity in a rat model of depression, accompanied by telomere elongation and reducing clinical symptoms [124]. In patients with bipolar disorder, telomere length correlated with duration of therapy [125]. Antipsychotic drugs may also have some positive influence on telomere length [126]. The modulation of intracellular Wnt/β-catenin or PI3K/Akt signaling pathways, the interaction with brain-derived neurotrophic factor and 5-HT, and antioxidant properties could represent possible mechanisms by which psychopharmacological drugs could modulate telomerase activity [127]. These pathways are functionally-relevant downstream drug effects and are also activation pathways for telomerase, suggesting a potential (yet unproven) mechanism by which these drugs may mediate neurogenesis via telomerae activation [38,128].

3. Telomerase as a Target for Cancer Treatment

There are several approaches for a telomerase-based gene therapy in the treatment of cancer. Cancer cells have high telomerase activity compared to most other cells [129]. The restriction of hTERT is a potential therapeutic option because telomerase complex components are up regulated in most tumor cells. Moreover, telomerase is a good target for cancer therapy because most somatic cells have no or only low level telomerase activities. Thus, the selective inactivation of telomerase expression in cancer cells does not influence most healthy cells [96]. Different therapeutic approaches for telomerase-based treatment of cancer have been developed or are under investigation [96,130].

(1). Oligonucleotide inhibitors. Antisense oligonucleotides and chemically-modified nucleic acids have been shown to inhibit telomerase and to induce telomere shortening [131–135] associated with subsequent onset of senescence and/or apoptosis in cell cultures [134–137]. These inhibitors act directly or indirectly (by inducing apoptosis). Targets include the RNA template, hTERT protein, and associated proteins. For example, the thio-phosphoramidate oligonucleotide inhibitor imetelstat (by Geron Corporation, Menlo Park, CA, USA) targets the RNA template for hTERT by binding to the catalytic site of telomerase [138]. Imetelstat (GRN163L) was successfully tested for glioblastoma tumors [139]. This cancer type ensures that there is sufficient time to permit tumor growth and erosion of telomeres to critical levels that trigger cellular senescence. Clinical phase II studies are planned for breast and lung cancers. Significant side-effects were not observed and possible combinatory therapies with well-established regimes for myeloproliferative neoplasms and acute myeloid leukemia are under investigation [96].

(2). Small-molecule telomerase inhibitors. Small-molecule telomerase inhibitors have been identified in screens of chemical libraries or were synthesized based on the structure of natural telomerase inhibitors such as epigallocatechin-3-gallate (EGCG) [140–144]. Moreover, various targets with overlapping functions have been proposed such as the PI3K-Akt-mTOR pathway [145], which is often dysregulated in cancer. The mTOR inhibitor rapamycin was shown to inhibit telomerase activity [146–148] and to counteract carcinogenesis.

(3). Immunotherapeutic approaches. The active site of telomerase in cancer cells is a possible target to develop vaccines [149]. Adoptive cell therapy, with the use of high-avidity T lymphocytes reactive against telomerase, has successfully been used in adenocarcinoma mouse prostate mice, which develop androgen-independent prostate cancer [150]. Despite marked temporary autoimmune depletion of B cells as side effect this therapy was not associated with significant immunoglobulin decreases or infections. At least 23 clinical studies, summarized in [151] have investigated hTERT immunotherapy as anticancer strategy in melanoma, acute myeloid leukemia, glioblastoma, prostate, renal, pancreatic, hepatocellular, and non-small-cell lung cancer: 18 phase I/I-II studies [152–168], four phase II studies [169–172], and one phase III trial [173] with pancreatic cancer patients and GV1001. Median survival ranged from 88 to 450 days in non-responders and from 216 to >600 days in responders [157,158,163,166,170,172], with the pancreatic cancer patients showing best survival rates so far. In a phase I trial an hTERT-derived peptide was used as a vaccine in hepatocellular carcinoma patients, with the majority of patients showing recurrence up to 24 weeks after vaccination [174].

Possible reasons for the limited success in many studies are the development of self-tolerance, the limited size of the precursor T-cell repertoire, negative effects of immunosuppressive tumor microenvironment on T cells, and interindividual differences. Various improvements have been proposed for future studies. These include (i) stimulation of cooperation between CD8+ and CD4+ T cells, by immunization with both MHC class I and class II hTERT peptides, in order to expand the pool of persisting memory CD8+ T cells; (ii) limiting the development of immune-tolerance, by immunization with low affinity (mutant) MHC I hTERT peptides, to increase the efficacy of vaccination; (iii) limiting the development of immune-tolerance by parallel immunization with peptides derived from non-self antigens; and (iv) development of personalized approaches with a focus on patients with early stage diseases to avoid negative effects of immunosuppressive cancer microenvironments [151].

(4). Telomerase-directed gene therapy. The promoters for telomerase in cancer cells are targets for a tumor specific gene therapy that selectively kills cancer cells and leaves normal cells unharmed by expressing high concentrations of a therapeutic protein only in cancer cells. Adenoviruses are developed that (by use of the hTERT promoter) selectively replicate in cancer cells and, subsequently, kill the cancer cells [175]. Both cytotoxic gene therapy and oncolytic virotherapy approaches have been used to kill cells expressing telomerase and not killing healthy cells [176].

(5). Phytochemicals. A wide variety of chemical compounds that occur naturally in plants, or phytochemicals, have been suggested to inhibit telomerase activity in various cancers, summarized in [177]. The substances include allicin, an organophosphate derived from garlic [178]; curcumin, a phenol present in turmeric [178–185]; the flavonolignan silbinin; an organosulfur derived from cruciferous vegetables; epigallocatechin gallate (EGCG), a catechin in green tea [186]. Curcumin [181], genistein [187], EGCG [188], and sulforaphane [189] were tested in breast cancer cells and the non-malignant breast cell line. The mode of action is only partially known and encompasses inhibition of translocation of hTERT to the nucleus [179]; dissociation of Hsp-90 co-chaperone from hTERT [183]; and a decrease of hTERT expression or activity [180–182,190].

Despite extensive studies during prior years on the development of telomerase vaccines, telomerase inhibitors, and telomerase promoter-driven cell killing in oncology, only one therapeutic vaccine went all the way to the clinic (GV1001), and only one telomerase antagonist (imetelstat, GRN163L) is in late preclinical studies. However, numerous drugs with various other targets have been identified with additional off-target effects on telomerase activity. These include substances which act via downregulation of hTERT gene transcription: the tyrosine kinase inhibitors dasatinib, imatinib, gefitinib, and nilotinib [191–193]; the ubiquitin/proteasome pathway inhibitor bortezomib; the cytotoxic drugs 5-azacytidine [194], arsenic trioxide [195] and temozolomide [196]; the chemosensitizer suramin [197]; the non-steroidal anti-inflammatory drugs aspirin [198], indomethacin [198], and celecoxib [199]; the peroxisome proliferator-activated receptor (PPAR) activator troglitazone [200]; the histone deacetylase inhibitors romidepsin [201] and vorinostat [202]; and the mTOR pathway inhibitor rapamycin [203]. The DNA topoisomerase I inhibitor beta-lapachone [204] and the DNA crosslinker cisplatin [205] act via downregulation of hTR gene transcription. The circadian rhythm hormone melatonin downregulates both hTERT and hTR on transcriptional level [206]. Other substances inhibit telomerase activity by unknown mechanisms: perifosine [207], nimesulide [208], auranofin [209], pyrimethamine [210], azidothymidine [211], octreotide [212] and ofloxacin [213]. Quinacrine, bortezomib, etoposide, and doxorubicin directly target the telomere structure proteins TRF1, POT1, shelterin, and TNKS1 [214–217].

Various drugs proposed for skin cancer therapy, including tyrosine kinase and Wnt/β-catenin signaling inhibitors, also have inhibitory effects on telomerase [218–223]. This is not unexpected because telomerase enzyme activity can be post-transcriptionally regulated by the kinases c-Abl, protein kinase C, ERK1/2, and Akt. [224–234].

Blockade of the epidermal growth factor receptor might be effective in inhibiting telomerase activity of squamous cell carcinomas, which may result in suppression of tumor growth [235]. The recent finding of a germline mutation in the promoter of hTERT in a melanoma-prone family further suggests the importance of telomerase as an important target in skin cancer therapy [236]. Resveratrol, by contrast is, at least in mouse models, a potent chemopreventive agent against melanoma [237,238] but rather increases telomerase activity.

4. Advantages, Pitfalls, and Outlook

There are many advantages for using telomerase as an anti-cancer target. First, it is an essential and specific component for most cancer cells [129,138] and more widely expressed than any other tumor marker. Approximately 90% of all human cancers have elevated telomerase levels relative to normal cells. Second, telomerase is the most efficient mechanism for replicative immortality with only one (less robust) compensatory mechanism (ALT), which limits the risk for development of resistance to telomerase-based therapies. Third, the very low expression of telomerase in normal tissues, together with the longer telomeres in normal stem cells versus cancer cells, provides at least some degree of specificity with low risk of toxicity in normal cells [239], and limited risk in stem cells, provided that telomerase inhibition is limited in time.

There are also various pitfalls for a use of telomerase as a therapeutic target in cancer treatment. First, the anti-proliferative effects of telomerase inhibition are induced in cells with short telomeres only, which requires some time of tumor growth until the drug can be effective. Second, telomerase inhibition may elicit negative effects in highly proliferative cells which need telomerase for survival, namely, stem cells, etc. [240]. Despite shorter telomeres in cancer cells, these two points may restrict the therapeutic option for a narrow telomere length window. Moreover, it has been shown that stress induces a shift of telomerase from the nucleus to mitochondria, suggesting a telomere-independent physiological function and possible risks in long term inhibition of telomerase [16]. There are also caveats to the therapeutic strategy of senescence induction, per se. On one hand, both telomere-driven replicative senescence and stress-inducible senescence are tumor-suppressive [241–247]. On the other hand, senescence can be reversed by a process called crisis and then has pro-tumorigenic paracrine effects. By induction of telomere dysfunction and attrition chromosomal instability occurs and may result in activation of oncogenes and/or silencing of tumor suppressor genes, which may counteract the original therapeutic intention and cooperate to promote malignant transformation and drug resistance [248–252]. Finally, the currently available drugs and substances are often unspecific and have a wide variety of actions and different cellular targets that may counteract replicative immortality but may also exacerbate other cancer hallmarks such as chromosomal instability. Some studies are promising but also suggest that for complete eradication of all subpopulations of cancer cells a simultaneous targeting of several mechanisms will be needed. It is unlikely that a single target will provide lasting remission.

Approximately 5%–10% of cancer cells maintain their telomeres by ALT [28]. This process is only partially understood but may offer new therapeutic options by modulating the involved factors such as the shelterin complex or the telomere sequences themselves to induce telomere deprotection.

There are also a number of pitfalls in the treatment with telomerase-activating procedures or substances. Immortality is not intrinsically essential for malignancy [253]. However, an extended lifespan may accumulate rare genetic and epigenetic aberrations that can contribute to malignant transformation. Constitutive telomerase expression in mice increased tissue fitness and delay of aging at the expense of slightly increased cancer incidence [254–256]. In mice with cancer resistant backgrounds (by increased expression of tumor suppressors p16, Arf, and p53) transgenic telomerase expression extends lifespan by 43% [254]. Interestingly, the cancer-promoting activity in transgenic mouse models is not observed when telomerase is re-activated later in life.

In this context, a recently described methodological advance is of interest. Bernardes de Jesus et al. have designed a potential therapeutic approach in which telomerase is induced temporarily and selectively in old cells without promoting cancer growth. The special feature of recombinant adeno-associated virus (rAAV) vector is its 'mild' integration into the host genome. By use of rAAV vectors, which expressed the catalytic subunit of mouse telomerase (mTERT) it was integrated into the host genome at very low rates but, in mice, did not induce cancer growth. A possible explanation is the loss of the vector in rapidly proliferating cells such as cancer cells. Thus, this method might be used to treat age-related diseases, such as atherosclerosis or diabetes [257]. In short-lived organisms, such as mice, this strategy seems to be an excellent approach for a telomerase-based gene therapy. For long-lived organisms it is currently unknown if cancer can be promoted by rare integration events of constitutively-overexpressed hTERT [257].

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Abbreviations

The following abbreviations are used in this manuscript:

ALT	alternative lengthening of telomeres
CRP	C-reactive protein
hTERT	human telomerase reverse transcriptase
hTR	human telomerase RNA
IL-6	interleukine-6
LTL	leucocyte telomere length
mTERT	mouse telomerase reverse transcriptase
rAAV	recombinant adeno-associated virus
ROS	reactive oxygen species
TERC	telomerase RNA component
TERT	telomerase reverse transcriptase
VEGF	vascular endothelial growth

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