

# Telomerase Therapeutics for Degenerative Diseases

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**Abstract:** Telomerase is active in early embryonic and fetal development but is down-regulated in all human somatic tissues before birth. Since telomerase is virtually absent or only transiently active in normal somatic cells throughout postnatal life, telomere length gradually decreases as a function of age in most human tissues. Although telomerase repression likely evolved as a tumor suppressor mechanism, a growing body of evidence from epidemiology and genetic studies point to a role of telomerase repression and short telomeres in a broad spectrum of diseases: (a) Humans with shorter than average telomere length are at increased risk of dying from heart disease, stroke, or infection; (b) Patients with *Dyskeratosis congenita* are born with shortened telomeres due to mutations in telomerase components, suffer from a variety of proliferative tissue disorders, and typically die early of bone marrow failure; and (c) Individuals with long-term chronic stress or infections have accelerated telomere shortening compared to age-matched counterparts. Telomerase activation may prove useful in the treatment of diseases associated with telomere loss. While human cells dividing in culture lose telomeric DNA and undergo changes that mirror certain age- or disease-associated changes *in vivo*, telomerase transduced cells have extended replicative capacities, increased resistance to stress, improved functional activities *in vitro* and *in vivo*, and no loss of differentiation capacity or growth control. In addition, telomerase transduction *in vivo* can prevent telomere dysfunction and cirrhotic changes in liver of telomerase knockout mice. Thus, pharmacological activation of telomerase has significant potential for the treatment of a broad spectrum of chronic or degenerative diseases.

**Keywords:** Telomere, telomerase, disease, aging, *Dyskeratosis congenita*, therapeutics, gene therapy.

## TELOMERES AND TELOMERASE IN CELL AGING AND CANCER

Telomeres are essential genetic elements "capping" the ends of our chromosomes [1] (see review of Crabbe and Karlseder in this issue). They are maintained in immortal cancer cells and cells in the germ lineage by expression of telomerase [2, 3]. Telomerase consists of two core subunits: hTR, the human telomerase RNA component [4], and hTERT, the human telomerase reverse transcriptase [5]. In humans, telomerase is active in early fetal development, but is repressed in essentially all somatic tissues before birth [3, 6]. In postnatal somatic tissues, telomerase is repressed, or is present transiently or at very low levels, and telomeres gradually erode with time and cell division [7-11] (see review of Hahn in this issue). Eventual loss of telomere function on one or a few chromosomes triggers a complex response associated with damaged DNA, leading to loss of normal cell function, division capacity, and/or cell death [12, 13] (see review of Hazel *et al.* in this issue). This process of "replicative senescence" may play an important role in age-related diseases (e.g. cardiovascular diseases, stroke, macular degeneration, osteoporosis, joint disease), and in conditions such as viral infections or chronic stress (e.g. AIDS, liver diseases, and skin ulcers) [14-19].

Telomerase is constitutively active in the vast majority of biopsies from all cancer types studied to date. However, telomerase does not cause cancer [14]. This has been shown by experiments in which over-expression of telomerase causes no malignant changes in normal cells [20, 21], and by the fact that embryonic stem cells [22] and developing germ cells have activated telomerase, yet remain normal. Cancer is primarily characterized by abnormal cell growth caused by activation of oncogenes or loss of tumor suppressors that regulate cell growth and division. During early growth and expansion of mutated cells, telomerase is typically inactive and telomeres continue to shorten until telomerase is activated through one or more additional mutations, conferring immortality to the cancer. Thus, even though telomerase activation is important for cancer cell survival, and with long-term growth telomerase immortalized cells may acquire transforming mutations [23], telomerase activation in normal human cells is expected to improve their function without causing cancerous changes. In fact, telomerase activation could have the paradoxical effect of reducing the frequency of cancer if the beneficial effect of restoring normal function to aging cellular systems outweighs the risk of extending the lifespan of premalignant cells [23].

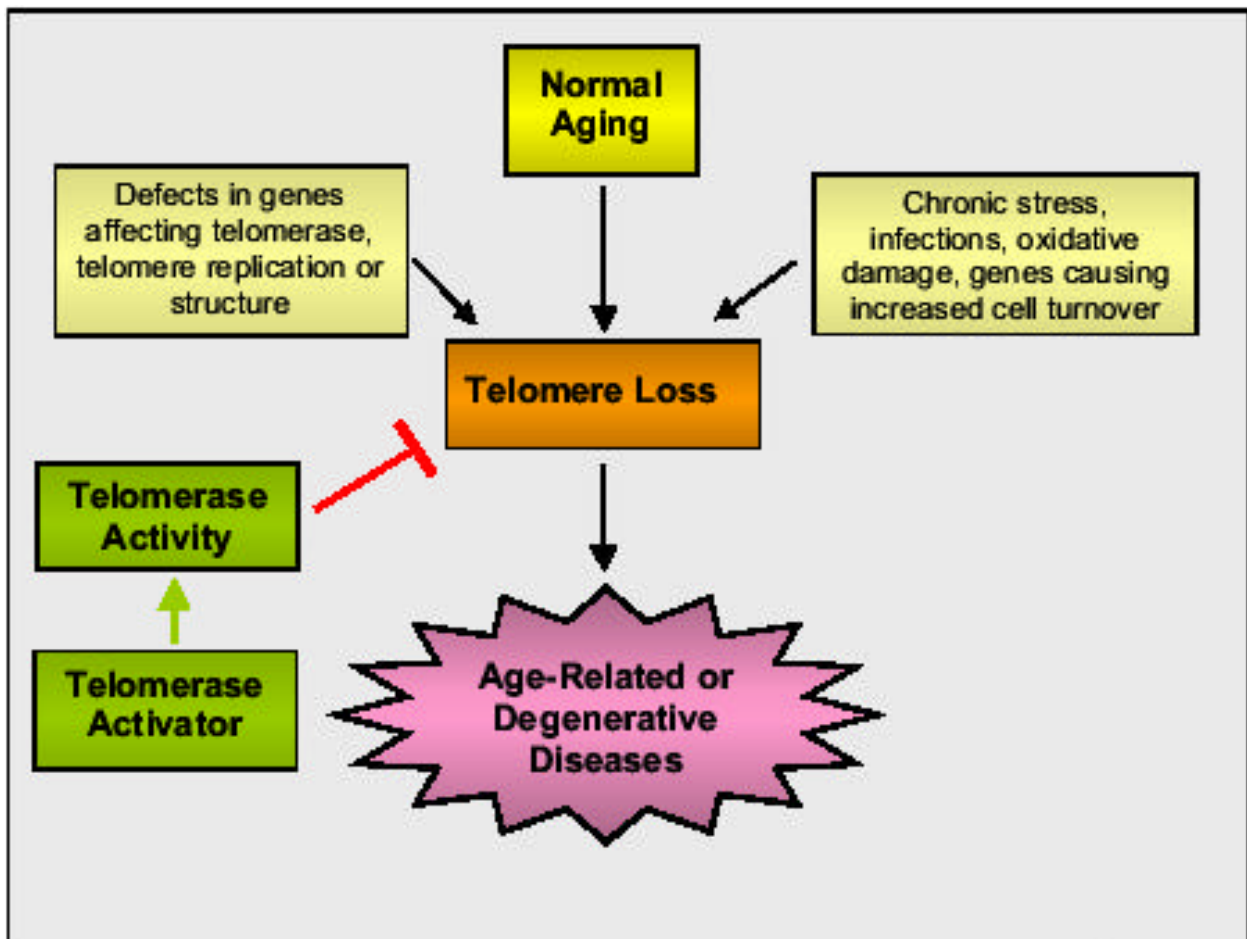
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## ANIMAL MODELS, HUMAN EPIDEMIOLOGY, GENETIC STUDIES, AND CHRONIC STRESS LINK SHORT TELOMERES TO DEGENERATIVE DISEASES

There are multiple lines of evidence suggesting that telomere loss may play a role in degenerative diseases. First, the telomerase knock-out (TKO) mouse has proven to be a useful model of human aging and disease (see review of Chang in this issue). Laboratory strains of mice have not been a good model of human aging and age-related disease, but their telomeres are much longer than those in humans. In later generations of inbred TKO mice, when telomeres shorten to lengths similar to those seen in aging humans, and in TKO mice crossed with mice harboring genes linked to premature aging in humans, a variety of diseases in proliferative tissues mimicking those in the humans are revealed for the first time [17, 24-28]. This suggests that short telomeres can link aging and disease in mice to the human state.

Second, in epidemiological studies, individuals with short telomeres in their blood cell DNA were found to be statistically at higher risk for stroke (cerebrovascular dementia), heart disease, and infections, compared to individuals with longer telomeres [29, 30] (see review of von Zglinicki and Martin-Ruiz in this issue). In the case of heart disease and infections, the risk factor for mortality in unrelated individuals over the age of 60 with short telomeres was increased 3.2-fold ( $p=0.008$ ) and 8.5-fold ( $p=0.015$ ), respectively [30].

Third, human genetic disorders such as X-linked or autosomal dominant *Dyskeratosis Congenita* (DKC) affecting the structure of hTR or telomerase-associated proteins, respectively, cause decreased telomerase activity in all cells throughout life (see review of Mason *et al.* in this issue). This leads to shortened telomeres at birth and rapid progression to failure in proliferative tissues such as bone marrow, skin, hair, nails, liver, and gut [31]. Other genetic disorders such as Down's syndrome [11],



**Fig. (1).** Schematic illustrating telomere loss with normal aging leading to age-related or degenerative diseases. Mutations in telomerase or genes involved in telomere replication or structure can cause accelerated telomere loss in all tissues during fetal development and/or in proliferative tissues throughout life, leading to early onset disease. Similarly, chronic stress from infection, oxidative damage, or other extrinsic (environmental) or intrinsic (genetic) factors leading to increased cell turnover or greater telomere loss per cell division, can also accelerate telomere loss and disease progression. In contrast, generalized or targeted telomerase activation can increase telomere length or slow the rate of telomere loss, and possibly prevent or delay the onset of disease.

certain forms of aplastic anemia [32], Fanconi's anemia [33], and Werner's syndrome [34] can also lead to shortened telomeres, accelerating aging and failure of proliferative tissues. In some cases (e.g. forms of aplastic anemic and Werner's syndrome), the genetic lesion has a direct impact on telomere replication either through telomerase or other aspects of telomere replication. In other cases the impact on telomere loss is not fully understood and may simply be a consequence of chronic genetic stress and increased cell and tissue turnover, accelerating the natural age-dependent loss of telomeres.

Chronic stress from extrinsic factors might also accelerate telomere loss. A recent study showed that telomeres in blood leukocytes tend to be shorter in women exposed to long-term stress, a condition associated with increased susceptibility to multiple diseases [35]. Chronic infection with HIV leads to accelerated telomere loss in CD8<sup>+</sup> T cells, and critical telomere lengths in these cells is associated with progression to AIDS [16, 36]. Similarly, chronic hemodynamic stress near bifurcations in arteries is associated with shortened telomeres in endothelial cells and atherosclerosis [37]. The relationship between telomere loss, normal aging and age-related or degenerative diseases, and the contribution of intrinsic and

extrinsic factors which can lead to accelerated telomere loss, is illustrated in (Fig. 1).

## EVIDENCE THAT TELOMERASE ACTIVATION HAS POTENTIAL FOR DEGENERATIVE DISEASES

In addition to the correlative data listed above linking telomere loss with disease in humans, there are a variety of experimental studies that directly support the potential of telomerase activation for the treatment of degenerative diseases. First, a variety of human cell types grown in culture exhibit little or no telomerase activity, gradual telomere loss, and a finite lifespan culminating in replicative senescence associated with loss of normal differentiated function [38, 18, 39, 40, 21, 41-43] (see reviews of Hahn and of Hazel *et al.* in this issue). These cellular changes in the laboratory dish have been linked to age- and disease- related changes *in vivo* [14, 44, 39]. Introduction of an active form of the catalytic protein component of telomerase, hTERT, by gene transfer into human cells typically increases telomere length, extends cellular lifespan, and restores (or prevents loss of) normal differentiated function. Moreover, such "telomerized" cells have normal growth control and generally show no signs of malignant changes [14, 40, 21]. In many cases, introduction of active

**Table 1. Cells that Respond to Telomerase Gene Transduction with Improved Replicative Capacity, Differentiated Function, and/or Resistance to Stress**

• Bone: Osteoblasts [60-62]
• Brain and the nervous system: <i>Neurons and neural progenitors</i> [49, 63, 64]
• Breast: Mammary epithelial cells [65, 66]
• Connective tissue: <i>Chondrocytes</i> [67]
• Endocrine system: Adrenocortical cells [68]
• Esophagus: Keratinocytes, squamous cells [69, 70]
• Eye: Retinal pigmented epithelial (RPE) cells [71], corneal keratocytes [72]
• Gum tissue: Gingival fibroblasts [73]
• Heart: Cardiomyocytes [45]
• Immune and hematopoietic system (normal): Cytotoxic T cells [74, 75, 15], Hematopoietic stem cells [76]
• Immune system (impaired, e.g. HIV/AIDS): <i>Cytotoxic T cells</i> [16, 77]
• Liver: Hepatocytes [78], stellate cells [79, 80], cholangiocytes [81],
• Muscle: Skeletal myocytes [82]
• Ovary: Surface epithelial cells [83]
• Pancreas: Ductal stem or precursor cells [84]
• Uterus: Endometrial glandular cells [85], stromal cells [86] and myometrial cells [87]
• Skin: Keratinocytes [88], fibroblasts (reviewed in [14], microvascular endothelial cells [89, 90], lymphatic endothelial cells [91, 92], melanocytes [93]
• Vasculature: Brain [94], retinal [95] and microvascular endothelial cells, smooth muscle cells [96]
• Other: Mesenchymal [61, 97], adipose [98] and bone marrow stromal [99] stem cells; bone marrow endothelial cells [100]

**Table 2. *In vivo* Models in which Telomerase Activated (hTERT Transduced) Cells have Improved Function over Control Cells**

•	Wound healing (human skin reconstitution in mice): <i>Human fibroblasts</i> [43] and <i>keratinocytes</i> (Harley, C.B. unpublished data)
•	Neovascularization (normal skin or ischemic hind limb salvage in mice): <i>Human endothelial or endothelial progenitor cells</i> [101, 102]
•	Bone formation (human cells or bone fragments injected into mice): <i>Human osteoblasts or mesenchymal stem cells</i> [61, 103]
•	Dentin formation (rat cells into rat): <i>Odontoblasts</i> [104]
•	Cancer immunotherapy (human melanoma in mice): <i>Human cytotoxic T cells specific for the implanted tumor cells</i> [105]

telomerase also increases the capacity of cells to withstand stress due to high or low levels of oxygen, toxic molecules, or abnormal growth conditions [45-49] (see review of Chung *et al.* in this issue). These observations are supported by the loss of stress resistance in telomerase-inhibited tumor cells and in the TKO mouse [26, 47, 50]. The ability of telomerase activation to increase stress-resistance in normal cells independent of cell division is important as it suggests that even non-dividing cells will benefit from telomerase activation. Examples of human cells responding positively to telomerase gene transduction are listed in Table (1). This list includes 19 different tissue systems and over 35 cell types.

The second line of research linking telomere loss to cellular aging and disease comes from *in vivo* studies in which hTERT-expressing cells are injected into rodent models to assess functional capacity (Table 2). In these 5 model systems of tissue repair or regeneration, including a model of cancer immunotherapy, normal cells which have been transfected with active telomerase form functional tissue more readily than their normal (untransfected) counterparts.

The third line of research supporting telomerase activation for the treatment of disease involves injection of a telomerase gene into telomerase knockout mice having short telomeres and compromised livers. Restoration of telomerase activity in the liver of these mice by mTR gene therapy prevented lethal loss of liver function upon exposure to toxic molecules or upon repeated partial hepatectomy [25]. In subsequent work by Rudolph and coworkers [17], it was shown that even though the absence of telomerase reduced the frequency of cancer in these mice, the impact of impaired tissue function due to telomere loss had a more dominant effect on survival. This suggests that the survival benefit of telomerase activation in the setting of compromised tissue homeostasis due to critically shortened telomeres should outweigh the potential risk of tumor promotion, consistent with arguments made previously [14].

### POTENTIAL FOR TELOMERASE ACTIVATION IN DISEASE THERAPY

Telomerase activation, by increasing telomere length or slowing the rate of telomere loss, should

delay or prevent the onset of degenerative changes in tissues caused by critical telomere loss (Fig. 1). Multiple approaches to telomerase therapeutics for degenerative disease are possible. Telomerase (hTERT) gene therapy could be utilized *in vivo* in either a targeted or non-targeted manner. Alternatively, cells could be removed from a patient and modified *ex vivo* with telomerase before re-introduction. Different gene therapy vectors (e.g. non-integrating or integrating vectors, with conditional or non-conditional expression) could be utilized to achieve shorter- or longer-lasting, or controlled expression of hTERT. This could achieve some level of control over the extent or duration of telomere elongation. Finally, allogeneic cells with hTERT-extended lifespans, with or without additional genetic modifications, could be used in an allo-transplant setting.

Although cell and gene therapy for the treatment of degenerative diseases remains a viable approach for telomerase activation, the ideal therapy would be a safe and effective small molecule activator of endogenous telomerase. A small molecule drug has the advantage that it is simpler to manufacture, qualify, distribute, and control, and does not carry the same level of risk for genetic modification to cells. Based on the research reviewed above, a small molecule telomerase activator could find utility in treatment of essentially all age-related diseases that involve reduced proliferative capacity or sensitivity to stress related to lack of telomerase activity or shortened telomeres. Table 3 provides a partial list of diseases that should respond to a telomerase activator drug. There is a precedent, at least conceptually, for the benefit of small molecule activators since the hTERT promoter is known to respond to estrogen [51-53]. We are engaged in efforts to discover and develop novel telomerase activators and anticipate that such drugs will be useful in the treatment of a broad range of chronic diseases.

### IMPLICATIONS REGARDING TELOMERASE THERAPEUTICS FOR CANCER

Since telomerase is repressed in most normal cells while playing a critical role in all tumor types studied to date, it is no surprise that telomerase is keenly targeted for cancer therapy [54]. The first clinical cancer studies based upon telomerase capitalize on the selective expression of hTERT as

**Table 3. Potential Uses of a Small Molecule Telomerase Activator**

• <b>AIDS:</b> Improved cytotoxic T cell elimination of HIV-infected CD4 cells
• <b>Cardiovascular and heart diseases:</b> Reduced ischemic damage, improved neo-vascularization
• <b>Chronic ulcers:</b> Improved wound healing
• <b>Joint diseases:</b> Improved cartilage production
• <b>Infections in the elderly:</b> Improved overall immune response
• <b>Liver disease:</b> Improved hepatocyte growth and resistance to stress
• <b>Macular degeneration:</b> Improved RPE cell function; reduced angiogenesis
• <b>Osteoporosis:</b> Improved osteoblast function and bone generation
• <b>Stroke and neurodegenerative diseases:</b> Reduced ischemic damage and increased resistance to neurotoxins (e.g. amyloid)

a pan-tumor-specific antigen for therapeutic vaccines. Phase I/II trials to date show a strong hTERT-specific immune response, preliminary signs of efficacy, and no indication of toxicity to normal tissues or stem cells [55] and Okarma T., AACR Special Conference on the Role of Telomerase in Cancer, Nov., 2004). A number of viral and gene therapy-based strategies are in preclinical development utilizing the selective activation of the hTERT promoter in tumor cells to trigger cell killing [56, 57]. In our pursuit of a direct telomerase inhibitor, we are now in IND-enabling development of GRN163L, a highly potent and specific lipid-conjugated thio-phosphoramidate oligonucleotide that binds the template region of hTR and acts as a direct enzyme inhibitor. This molecule, an analog GRN163 [58], has demonstrated efficacy in a variety of animal models of human cancer and has attractive pharmacokinetic and pharmacodynamic properties (Harley, C.B., AACR Special Conference on the Role of Telomerase in Cancer, Nov., 2004). Other strategies to kill telomerase-positive tumor cells have been reviewed elsewhere [59].

The implications of telomerase activation in the context of cancer initiation or progression has been discussed in depth previously [14]. A simplified approach to this question is illustrated in Fig. (2). For non-cancer patients suffering from degenerative disease or chronic conditions caused in part by telomere-dependent cell senescence, pharmacologic activation of telomerase should have relatively rapid benefit. Premalignant cells present in the patient should also have their telomeres extended and an extended lifespan could permit them to acquire additional mutations. However, most evidence suggests that critically short telomeres contribute to tumor initiation, and with immune and other normal cells benefiting from telomerase activation, it is possible that the net effect of telomerase activation in such patients could be tumor suppression. If the patient already has cancer, it is likely that the tumor is already telomerase positive, and thus the activator is not likely to impact tumor progression.

## CONCLUSIONS

Telomerase activation is a novel and attractive approach for the treatment of degenerative diseases afflicting elderly individuals and those with chronic conditions or infections that lead to accelerated cellular aging and loss of tissue homeostasis. The role of telomerase in conferring increased resistance to stress expands the potential of a telomerase activator beyond dividing cells to non-dividing tissues such as heart and brain. Although telomerase activation is associated with cancer progression, telomerase is not an oncogene, and in normal human cells and tissues, controlled telomerase activation with a small molecule activator should not impose an unacceptable cancer risk.

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