Telomeres and Cancer: A New Approach to Therapy

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As an organism ages, its body functions decline and it is more susceptible to disease and injury. By understanding how aging occurs, age-related diseases and pre-mature aging that affect the lives of many people may be cured. However, aging is a complex process and our current knowledge about the mechanism of aging is very limited. There are a few generally accepted mechanisms:

- damage to DNA, protein, and other cellular components by oxidation and free radicals
- cellular senescence
- environmental and dietary factors

Aging is likely the combined result of all these factors. With the recent advances in the field of telomere biology, cellular senescence has been proved to play an important role in aging. This knowledge may lead to the development of therapies against age-related disease. At the same time the relation between cancer and the telomere has been elucidated and the development of new anti-cancer therapies is underway.

Cell senescence and the telomere

The Hayflick limit indicates the number of cell cycles that a cell is capable of going through. When the Hayflick limit is reached, the cell will enter cellular or replicative *senescence*, which is a state of irreversible growth arrest [1,2]. Senescent cells have an altered transcription profile, which leads to changes in gene expression. Consequently, the morphology and function of these cells are different as well[1]. As an organism ages, senescent cells accumulate and the normal function and architecture of a tissue may not be maintained[2]. Senescent cells can also secrete factors that destroy tissue integrity as they stimulate other cells to proliferate[3]. This destruction of tissue may lead to the malfunction of organs and, eventually, to a decline in the function of body systems. For example, senescent fibroblasts are capable of degrading their extracellular matrix and can adopt a new profile of collagen secretion. These changes in fibroblasts give rise to the characteristics associated with aged skin[4]. Moreover, there is evidence showing that senescent cells secrete promoters of cancer progression. One example of this is the stimulation of *preneoplastic* (precancerous) and *neoplastic* (cancerous) epithelial cell growth both *in vitro* and *in vivo* by senescent fibroblasts. In contrast, when co-cultured with normal epithelial cells, the senescent fibroblasts did not stimulate the normal cells to grow[3].

Cells enter cellular senescence as the result of a dysfunctional telomere[3]. The telomere is a structure composed of DNA and protein. Telomeres are made up of the repetitive sequence (5'-TTAGGG-3') and a single-stranded 3' overhang. The length of the repeats varies among species and cell types. By interacting with telomere-associated proteins, the telomere forms a loop, called the t-loop, at the ends of linear chromosomes. Since the overhang is buried inside the loop, the ends will not be recognized as a break in the double strand[2]. Without telomeres, chromosomes are prone to degradation, recombination and fusion by DNA repair mechanism[5]. When the t-loop structure is disturbed, the growth of the cell will be arrested and the cell cycle checkpoints p53 and pRB are activated[2]. Once the checkpoints are activated, there are three

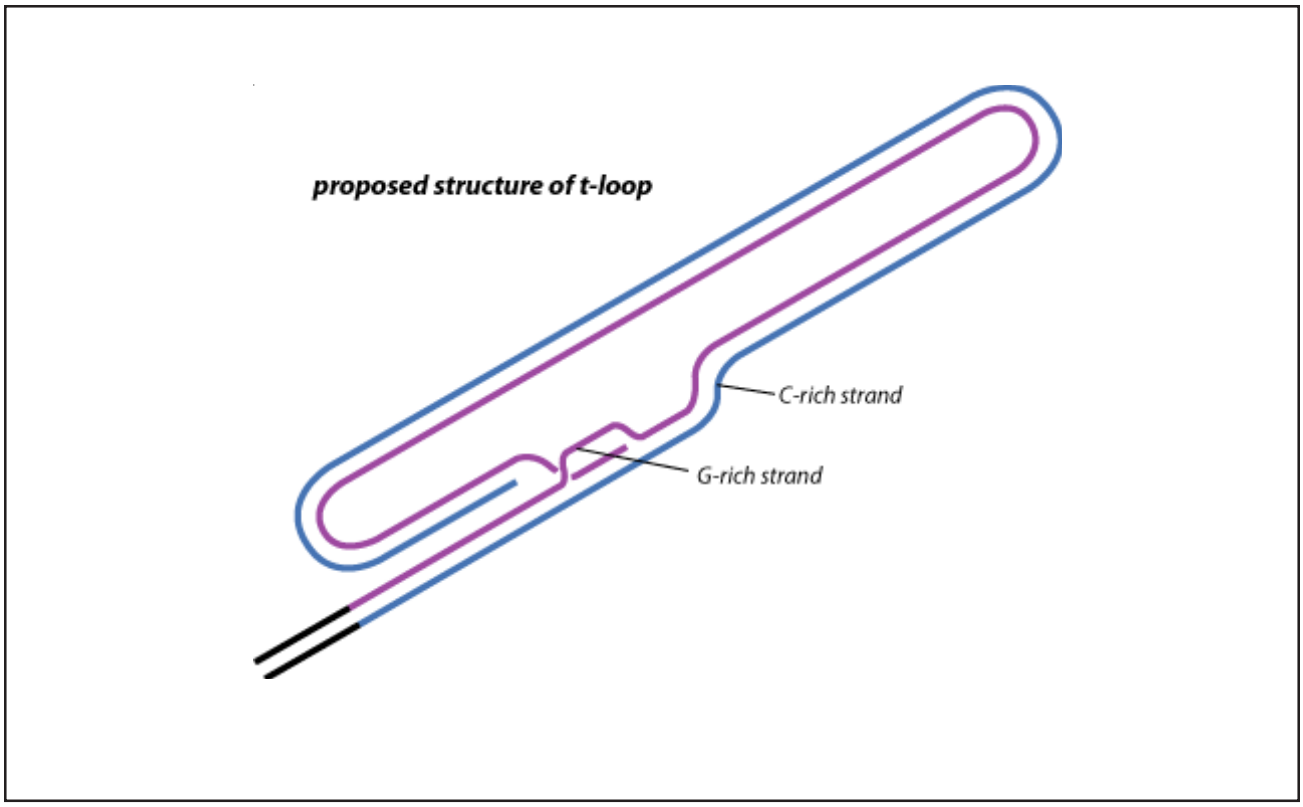

Figure 1. *The proposed structure of the t-loop, which is responsible for the stability of the chromosome.*

possible consequences for the cell:

(i) if the cell cannot bypass both checkpoints, the growth of the cell is arrested permanently and cell senescence occurs

(ii) if only the pRB checkpoint is bypassed, then intact p53 checkpoint will cause apoptosis

(iii) if both checkpoints are bypassed, the cell may continue to grow indefinitely, resulting in genomic instability[2].

DNA damage, malfunctioning of telomere-associated proteins, and natural telomere shortening may all lead to telomere dysfunction[2]. During normal DNA replication, between 50 and 200 base pairs at the 3' end of a chromosome are not replicated. Therefore, after each round of division, each chromosome has lost part of the telomere[3]. After many divisions, when only 4 to 6 kb of telomere remains, the telomere is too short to form a t-loop and the cell may senesce by activating the cell cycle check points[3]. Since telomere erosion happens as an organism ages, the telomere is known as the "mitotic clock" that is set for the onset of senescence[6]. In addition, telomere shortening can be accelerated by external factors such as damage by free radicals and oxidative stress. These factors usually cause a single-strand break in telomere DNA. When the cell divides, telomere base pairs distal to the break will be lost[7].

Telomerase and immortal cells

Although cellular senescence is the fate of most somatic cells, there are cells that will not become senescent and will continue to divide indefinitely. These cells include germ-line cells, hematopoietic stem cells, continuously regenerating cells in intestine crypt and skin, and activated lymphocytes[8]. In these cells, an enzyme called telomerase is activated to extend telomeres and to prevent senescence[2]. Human

telomerase consists of a RNA subunit (hTR) and a protein subunit (hTERT) [9]. The RNA subunit mediates the binding of the 3' overhang to the enzyme and contains the template 5'-CUAACCCUAAC-3', which codes for the telomere sequence [9,10]. The protein subunit is a reverse transcriptase [9]. The observation that cells with activated telomerase can proliferate indefinitely make telomerase a popular target for studying the mechanism of aging. Immortal cell lines can be created by activating telomerase in cells that normally have inactive telomerase. Bodnar and her colleagues *transfected* (inserted isolated nucleic acid into a cell via a viral carrier) foreskin fibroblasts with hTERT. Normally, only the RNA subunit (hTR) is expressed in fibroblast and telomerase is inactive. The transfected cells with both the RNA subunit and reverse transcriptase (hTERT) were capable of going through 20 more cell cycles than normal without changes in phenotype or malignant transformation [11]. This experiment proved that telomere length controlled the occurrence of cellular senescence and affected the lifespan of the cells. In another study, transfecting senescent fibroblasts with hTERT, reverted their gene expression and phenotypes to that of pre-senescent fibroblasts. When these transfected cells were transplanted on to an immunocompromised mouse (a mouse that will not reject foreign tissue), a piece of human dermal tissue without aging characteristics was formed [4]. This experiment suggested that by introducing telomerase into cells, we may be able to reverse the effects of aging and treat age-related disease[12].

Activation of telomerase to extend telomere length is not the only way to restore senescent cells' ability to divide. Slowing down the rate of telomere erosion can also extend the lifespan of a cell[13]. Two of the telomere-associated proteins, TRF1 and 2 (telomere repeat factor 1 and 2), can inhibit telomere elongation by looping the telomere and preventing telomerase from binding to the telomere[14]. When these two proteins are over expressed in cells with active telomerase, the telomere erosion still occurs despite the presence of telomerase. By inhibiting the binding of TRF1 to the telomere, telomerase can access the telomere and telomere elongation occurs[15]. Therefore, for cells with active telomerase that still encounter the problem of telomere shortening (for example, activated lymphocytes) inhibiting the expression or the telomere-binding ability of TRF can prevent them from entering senescence[8,13]. This study suggested that extended cellular lifespan can also be achieved by manipulating other telomere-associated proteins[16].

Treating premature aging and age-related disease

According to the studies discussed above, there are many possibilities for treating pre-mature aging and age-related disease, delaying the onset of aging, or even reversing the aging process. In theory, this can be done by replacing old tissues with new tissues that are constructed from immortal cell lines or by direct transplant of immortal cells onto aging tissues. The immortal cells will gradually outlive the aged cells and the tissue will consist mostly of immortal cells[12]. For example, the telomeres of the endothelial cells of aorta shorten with age. Since these cells are at the site where blood flow is generated, individuals whose aortic endothelial cells are worn out are at high risk of atherosclerosis. Therefore, replacing aged endothelial cells with immortal cells may prevent atherosclerosis[3].

Clinical studies examining whether replacing senescent cells can cure age-related disease are currently underway. These include studies on the relationship between Alzheimer's disease and senescent astrocytes and microglial cells, as well as between the decline in cornea function and senescent corneal epithelial and endothelial cells [12,17]. The rate of aging in patients with pre-mature aging disease may be decreased by activating telomerase in the cells of the patients. Werner syndrome (WS) is one such disease with symptoms similar to those of normal aging, but manifested at an accelerated rate. Studies show that the over-expression of the protein subunit (hTERT)

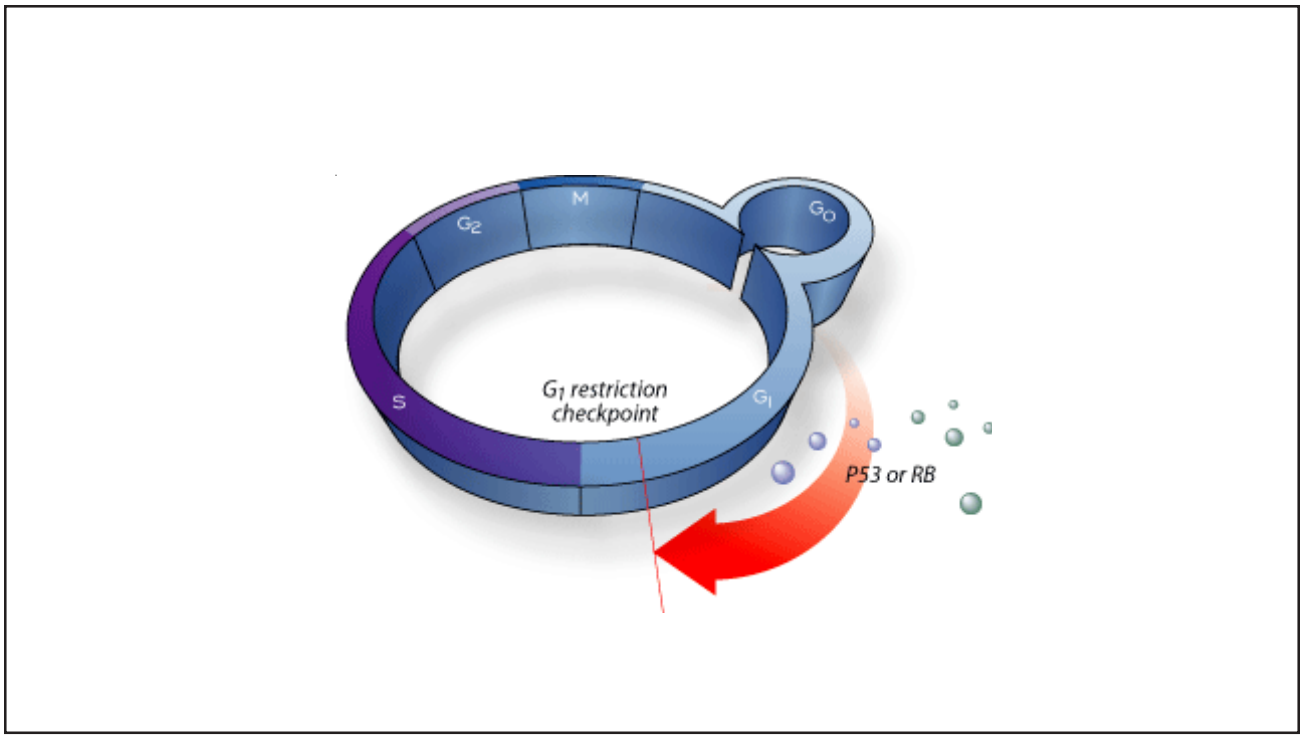

Figure 2. *The presence of p53 or Rb in the cell causes the cell cycle to stop at the G1 checkpoint.*

in WS cells increases telomerase activity, prevents telomere shortening, and decreases sensitivity of the cells to oxidative damage, all of which help to slow cell aging. Telomere shortening is also associated with other premature aging diseases such as Bloom syndrome and Down syndrome. However, the effectiveness of telomerase activation in treating these diseases has not yet been studied[18].

It is generally accepted that cell senescence is a mechanism designed to prevent cancer. A cell must have mutations in several *oncogenes* (genes that code for proteins which are involved in cell cycle regulation) and tumor suppressor genes before it can be transformed into a cancer cell. These mutations are accumulated over time. Cell senescence arrests the growth of aged cells and prevents further mutation accumulation in these cells, minimizing the chance for them to become malignant[6]. Re-activating growth in these cells may result in aged cells having more time to acquire mutations that are necessary for malignant transformation. In fact, Wang *et al.* (2000) showed that transfecting human mammary epithelial cells (HMEC) with hTRET not only led to the immortalization of the cells, but also to the activation of the oncogene, *c-myc*. The level of c-myc protein in these immortal HMEC was comparable to that of breast cancer cells. Therefore, transfecting cells with hTERT to immortalize them may lead to cancer cells[19]. This possibility raises safety concerns about using immortal cells for therapeutic purposes.

Treating cancer

Telomerase is expressed in many cancer cells but not in normal cells. This makes the enzyme a marker for cancer diagnosis as well as a target for the study of inhibiting tumor growth[8]. Inhibition of telomerase in cancer cells causes telomere erosion in these cells and leads to size reduction of the tumor by arresting the growth of cancer cells or by inducing *apoptosis* (a form of cell death). Inhibition of telomerase can be achieved by targeting different parts of the enzyme. For example, by using *antisense oligonucleotides* (synthetic fragments of RNA or DNA that specifically

bind to their complementary messenger RNA) that target the RNA template in the RNA subunit (hTR), the reverse transcription of the telomere repeat sequence can be inhibited. A few oligonucleotides are now under clinical trial[9]. Antisense transcripts that are targeted to bind to the region of the RNA subunit will disturb the binding of telomerase to the telomere thus inhibiting telomerase function[9]. The Introduction of a mutant form of the protein subunit (hTERT) that does not have catalytic activity into cancer cells is another method that may be used. These mutants compete with functional hTERT to bind to the RNA subunit of telomerase and inhibit telomerase activity. Without active telomerase, the telomeres in cancer cells were lost and the cells eventually died. One problem with telomerase inhibiting therapy is the presence of a lag between the administration of the inhibitors and the appearance of expected effects in the tumor. The end result of apoptosis and growth arrest can only be seen when the telomeres are shortened significantly. So, if the original cancer cells contain long telomeres, the effects of the inhibitors will be slow to appear[20].

Another possible anti-cancer therapy is the induction of an immune response that kills the tumor cells. Using the protein subunit of telomerase (hTERT) as an antigen to activate T cells will give rise to T cells that are specific for killing cells with high levels of hTERT expression[8]. Since most normal somatic cells do not express hTERT, tumor cells with high level of hTERT are the preferential target for these T-cells. Telomerase can be a marker for cancer diagnosis in addition to being a good therapeutic target. Using telomerase as a marker requires only a small amount of sample (a few cells) but it can provide information on the stages of cancer progression. In bladder, colon, lymphoid and prostate cancer, telomerase has been proven to be an apt diagnostic marker[21].

A recognized problem of targeting telomerase as anti-cancer therapy is the possible lethal effects on normal cells that express telomerase[8]. It is likely that normal cells with active telomerase will be killed in addition to the cancer cells. This can affect other body systems and have serious consequences for the organism. For example, if activated T-lymphocytes expressing hTERT become the target of these anti-cancer therapies, the T-cells may be killed and immune system may be compromised. Further studies on telomerase inhibitors are needed before these therapies can be administered in clinical trials. Another problem encountered with this method of treatment is that not all tumor cells require telomerase to proliferate. Therefore, these therapies could not be used to treat all types of cancer cells[22].

Ethical issues

For the time being, the idea of utilizing telomere and telomerase technology to postpone aging and to treat age related disease is still very young. However, it is impossible to ignore the ethical issues associated with this developing technology. One issue is the potential exhaustion of limited resources caused by extending the average human lifespan. Although having a longer lifespan would be beneficial to the individual, it might create problems for the human population collectively[23].

However, telomere biology may provide a method for replacing aged organs or tissue with less ethical concern than using embryonic stem cells. Resetting telomere length in senescent cells can produce young cells and tissues that can be transplanted into the patient, replacing the older tissues. This avoids the ethical and religious controversy that arises from the alternative of cloning the patient to create an embryo, then killing the embryo to harvest embryonic stem cells, which would then be transplanted into the patient to repair the aged tissues. The same ethical and religious controversies do not arise around the use of genetically manipulated senescent cells.

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