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The Dichotomic Role of Telomere Shortening- A Review.

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ABSTRACT

Telomeres from the ends of human chromosomes. Telomeres shorten with every sphere of organic process and this mechanism limits proliferation of human cells to a finite range of cell divisions by causing replicative senescence, differentiation, or programmed cell death. End shortening acts as a growth suppressor. However, as a drawback, there's growing proof indicating that end shortening conjointly limits vegetative cell function, regeneration, and organ maintenance throughout ageing. Moreover, end shortening throughout ageing and illness is related to increased cancer risk. This review puts the spotlight on the importance of telomeres in human health and aging and summarizes potential lifestyle factors that will have an effect on health and longevity by shifting the rate of telomere shortening. Recent studies indicate that end length, which can be affected by several lifestyle factors, will have an effect on the pace of aging and onset of age-associated diseases. This review highlights the dichotomic role of telomeres shortening and describes the lifestyle factors which may affect telomeres, human health, and aging.

Keywords: aging, cancer, lifestyle, regeneration, telomere.

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INTRODUCTION

Telomeres are the complex of protein and DNA, comprised of 5'-TTAGGG-3' repeating sequence. They protect the DNA ends of chromosomes and maintain their length, which is requisite for cell viability and genome stability (Sandin et al., 2014, Palm et al., 2008). Telomere end has a 3'G-rich single-stranded overhang which forms a T-loop by folding back on itself and 3'overhang enters the DNA strand and forms a D-loop. T-loop and D-loop formation stabilizes the chromosomes end (Palm et al., 2008).

Telomerase is a ribonucleoprotein complex, which, is composed of two copies of Reverse transcriptase (TERT), RNA component (TERC), dyskerin (a protein encoded by the gene DKC1) and other proteins like NHP2, NOP10, GAR1 which are required for complex stabilization (Nakamura et al., 1997, Jiang et al., 2007, Blasco, 2005). TERC acts as a template for the addition (de-novo) of TTAGGG sequence to chromosomes end (Blasco, 2005). During embryogenesis, telomerase is active in humans. It only remains active only in germ cell lines, few stem cell lines and progenitor compartments in adults. It also gets activated in human cancer cells (Jiang et al., 2007).

Shelterin, a protein complex which is also known as telosome, is required for the cap formation (T-loop and D-loop) to protect the DNA ends of chromosomes. It contains six subunits: TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 (Liu et al., 2004, de Lange, 2005). TRF1 and TRF2 bind and recruit the other shelterin proteins and many other proteins to double-stranded repeat sequence of telomeres. TRF2 regulates the formation of single-stranded 3'overhangs to facilitate the chromosome end protection (Hockmeyer et al., 2005). Both TRF1 and TRF2 have a common domain structure: N-terminal TRFH domain and C-terminal SANT/Myb domain (de Lange 2005). POT1 contains two terminal folds that are highly specific for 5'-TAGGTTAG-3' sequence of telomere 3'overhang (Lei et al., 2004) which promotes the formation of T-loop to facilitate the end protection (Hockmeyer et al., 2005). TPP1 interacts with POT1 and TIN2 and also functions for the recruitment of telomerase to the telomere (Wang et al., 2007, Xin et al., 2008). Since RAP1 have telomere binding ability so it interacts directly with TRF2 for telomere localization (Xin et al., 2008, Palm et al., 2008).

TELOMERE SHORTENING

During each round of cell division, there is a loss in length of telomere which is called telomere shortening. Two factors are mainly responsible for the telomere shortening: End-replication problem of DNA polymerase and suppression of telomerase expression. There is a loss of 50-100 bps occurs with each cell division in fibroblast telomere in humans. The telomeres lose their capping function when they become critically shorter in length and become dysfunctional which causes their recognition as DSBs and DNA damage. This induces two DNA damage checkpoints: the first checkpoint is the First mortality stage (M1) in which permanent cell cycle arrest occurs. This checkpoint is called senescence and it depends on the tumor suppressor gene p53 activation. Cells which have mutant p53 can bypass this checkpoint and start proliferating instead of having short and dysfunctional telomeres. Second checkpoint, second mortality stage (M2) is called crisis, which occurs due to further telomere shortening which causes a decrease in telomere function. This checkpoint does not depend on the p53 activation and there is a massive chromosomal instability and cell death (Jiang et al., 2007).

In humans, the length of telomere decreases in the rate of 24.8-27.7 base pairs per year (Shammas, 2011). After 50-70 cell divisions, human fibroblast cells reach senescence (Jiang et al., 2007).

TELOMERE SHORTENING BY LIFESTYLE FACTORS

It has found that telomere shortening may be caused by several lifestyle factors. Smoking, obesity, lack of exercise, and consumption of unhealthy diet are the factors that may increase shortening of telomere which causes illness and premature death. Increased shortening of telomeres can cause early onset of severe age-related diseases like heart diseases (including coronary heart disease and heart failure), increased risk of cancer and diabetes, osteoporosis and others (Shammas, 2011).

END-REPLICATION PROBLEM CAUSES TELOMERE SHORTENING

At the ends of telomeres, the incomplete replication of linear DNA occurs at every cell division. The DNA polymerase is unable to replicate the DNA at telomeres end efficiently due to the resection and fill-in

reactions which occur during the telomere leading strand synthesis (Maestroni et al., 2007). The leading strand is synthesized continuously and the lagging strands are produced as short discontinuous fragments of DNA called Okazaki fragments during the DNA replication. These discontinuous Okazaki fragments are synthesized by a very short RNA stretch called RNA primers. The RNA primers are replaced by DNA synthesis and the Okazaki fragments are ligated; this process is called maturation of Okazaki fragments which produces a continuous lagging strand. The removal of RNA primer during lagging strand synthesis causes potentially irreparable loss. There is a potential loss of less than two telomeric repeats on the lagging strand at each cell division since RNA primer contains 10 nucleotides in itself (Lundblad, 2012).

TELOMERASE DEFICIENCY SHORTENS TELOMERE

The telomerase deficiency, telomerase inhibition, and mutation in components of telomerase cause telomere shortening. Three human diseases are found to be associated with germline mutation the genes that code the two essential components: TERT and TERC of telomerase. Dyskeratosis cogentia (DKC), bone marrow failure, and idiopathic pulmonary fibrosis patients have been found to have a germline heterozygous mutation in the genes of telomerase (Garcia et al., 2007).

DYSKERATOSIS COGENTIA (DKC)

The patients with DKC, were found to have very short telomeres in length (Calado et al., 2009). A DKC patient is characterized by three signs: nail dystrophy, patchy skin hyperpigmentation, and leukoplakia (white patches) (Calado et al., 2009, Garcia et al., 2007). Some studies have shown mutations in TERC, NOP10, NHP2, and TERT. Homozygous mutations in TERT, NHP2, and NOP10 and heterozygous mutations in TERC were found when genetic screening was done (Calado et al., 2009). Dyskerin, a small nucleolar protein encoded by gene DKC1, binds to RNA (rRNA and TERC). Decreased levels of TERC have been seen in cell lines from patients with X-linked DKC which limits the length of the telomere. One copy of the X-linked DKC1 gene and mutated form of dyskerin was found in cell lines of males affected with DKC (Garcia et al., 2007).

MUTATED SHELTERIN COMPONENTS, FACTOR FOR TELOMERE SHORTENING

In the autosomal dominant DKC, mutations in TINF2 were found which caused telomere shortening (Calado et al., 2009). Approximately 50% of single-stranded TTAGGG repeat signal has found to be reduced by the induction and expression of dominant negative $TRF2^{\Delta B\Delta M}$ mutated allele (Steensel et al., 1998).

REPRESSION OF DNA DAMAGE SIGNALING IS REQUIRED FOR TELOMERE MAINTENANCE

Shelterin components TRF2 and POT1 play role in the repression of DNA damage signaling. TRF2 inhibits the ATM kinase activation at chromosome ends and the ATR kinase signaling is prevented by POT1. The end-replication problem causes DNA damage responses and repair pathways of telomeres end is repressed by shelterin complex. After the signaling of DNA damage, DSBs repair pathways get activated and cause chromosome fusions that are repressed by several factors to maintain chromosome ends. Two types of repair pathways: Homologous Recombination Repair (HRR) and Non-homologous End Joining (NHEJ). For the prevention of chromosome fusion, NHEJ needs to be blocked and to prevent the shortening of telomeres length, HRR is required to be controlled by mammalian telomeres. The ATM and ATR pathways can cause cell cycle arrest so they need to be blocked by them and they also need to prevent hypersection of chromosome ends (Doksani et al., 2014).

3'overhangs function as the telomerase priming site since telomerase is unable to act on the blunt ends and also 3'overhangs are considered for chromosome end protection. HR is repressed by the combined action of shelterin components: RAP1 and POT1 (Doksani et al., 2014). The NHEJ repair pathway is of two types: Classical or canonical NHEJ (C-NHEJ) and Alternative NHEJ (A-NHEJ).

C-NHEJ REPAIR PATHWAY AND ITS REPRESSION

C-NHEJ is a very fast process which operates with a half times on 10-30 minutes (Dueda et al., 2013). The very first step in the NHEJ repair pathway is the recognition of DSBs. It is considered that KU, a toroidal

protein, binds first when DSBs arise (Leiber et al., 2010). KU heterodimer binds to DSBs after their recognition, KU heterodimer is composed of two subunits: KU70 and KU80. After the binding of KU heterodimer, it acts as a scaffold for the recruitment of other required factors at the site of damage. These factors include DNA PKcs, XRCC4 DNA ligase IV, and XLF. The recruitment of DNA PKcs to the DNA is done by KU heterodimer which leads to the activation of DNA PKcs kinase activity. XRCC4 interacts with ligase IV to stabilize it and stimulate the ligation activity (Davis et al., 2013).

C-NHEJ can be repressed by the action of TRF2. TRF2 forms the T-loop to prevent the C-NHEJ. Recently, an iDDR domain of TRF2 is discovered and by virtue of which action TRF2 acts. The iDDR domain is the small region between the TRFH domain and SANT/Myb DNA binding domain and it interacts with MRN complex and BRCC6, a deubiquitylating enzyme. It prevents the 53BP1 loading at DNA damage sites. Through excessive resection induction, C-NHEJ can be repressed at telomeres and also at other sites in the absence of 53BP1 (Doksani et al., 2014).

A-NHEJ REPAIR PATHWAY AND ITS REPRESSION

A-NHEJ is recognized as the major source of instability of the genome. (Bennardo et al., 2008) It is the end joining of two non-homologous DNA ends. The first step involves the end resection, which is of two sub-steps: In the first sub-step, ssDNA short stretches are synthesized by the action of MRN complex and CtIP and then a more extensive resection is done by EXO 1. PNK is a polynucleotide kinase enzyme which phosphorylates the 5'ends by its kinase activity. PNK is recruited along with PARP1 and XRCC1/LigIII to the DNA ends. The incompatible DNA ends are ligated by the XRCC1/LigIII complex, which is regulated by MRN complex (Frit et al., 2014).
A-NHEJ repair pathway is promoted by CtIP and repressed by the KU heterodimer (KU70/80) (Bennardo et al., 2008).

Both C-NHEJ and A-NHEJ are considered as error-prone repair on two counts:

- They have no mechanism that ensures the original DNA sequence restoration in DSBs regions (Dueda et al., 2013).
- They can regulate the joining of two unrelated DNA molecules (Dueda et al., 2013).

CANCER AND ROLE OF TELOMERES SHORTENING

The abnormal and unregulated continuous proliferation of any type of cell in the body cause tumor growth in the body which leads to cancer. Cancer cells do not respond to the signals that regulate the behavior of normal cells due to which they grow and divide in an uncontrolled manner and spreads throughout the body by invading normal cells and organs. A tumor is of two types based on their spectrum: Benign and Malignant. A benign tumor does not invade other cells and surrounding tissues and it does not spread throughout the other parts of the body. A common skin wart is a type of benign tumor. A malignant tumor on the other side invades the nearby tissues and spreads by the circulatory and lymphatic system to the other parts of the body. Based on the cells they arise from, the tumors are of three types: carcinomas, sarcomas, and lymphomas (leukemias). The cancer-causing substances, called carcinogens are: chemical agents, radiation and viruses and these carcinogens act by causing DNA damage and mutations. The cancer is a multistep process which begins with the mutation that causes abnormal proliferation and formation of a tumor, then the tumor progression leads to cancer. (29)
Telomere shortening causes senescence and apoptosis of cells, but in case of cancer, tumor cells survive senescence and keep on proliferating. There are two factors: reactivation of telomerase and alternative lengthening through which tumor cell bypass telomere shortening.

REACTIVATION OF TELOMERASE

There are 85% cases in which TERT component of telomerase is reactivated. It was observed that TERT colligates NFκB promoter, IL-6, TNFα and these are the critical factors for cancer progression (Akincilar et al., 2010).

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NFκB

It regulates both types of immunity: innate and adaptive means it is requisite for the defense of hosts. The expression of target genes, essential for cell proliferation, survival, and differentiation, is induced by the active form of NFκB (Akincilar et al., 2010).

PI3K-AKT PATHWAY

Cellular events such as proliferation and differentiation of cell that are important in tumorigenesis are regulated by PI3K-AKT pathway. It was observed that receptor tyrosine kinase and VEGF that are controlled by PI3K in ovarian cancer cells elevates the expression of TERT (Akincilar et al., 2010).

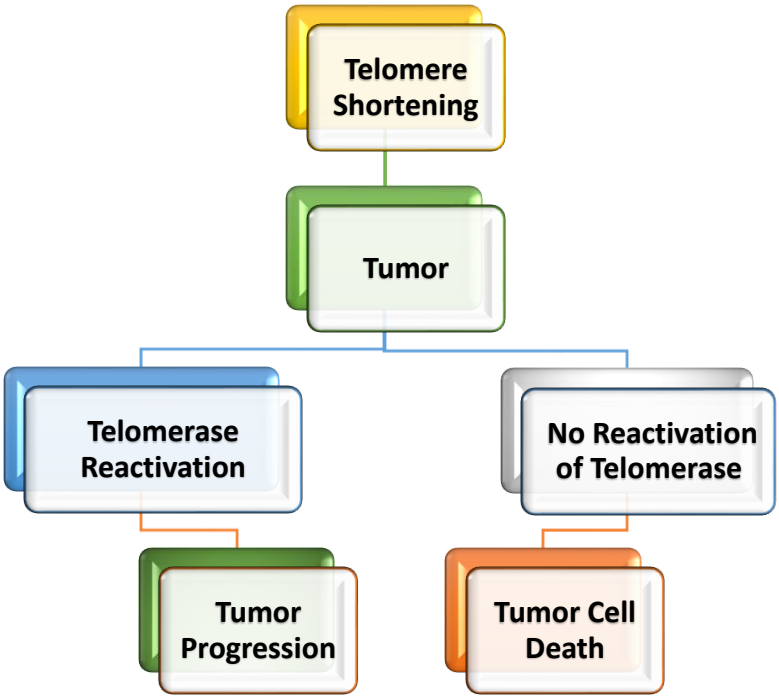

Figure 1: Effect of telomerase reactivation on the growth of tumor.

C228T AND C250T

ETS binding sites are created by the mutation in C228T and C250T in TERT promoter (Akincilar et al., 2010). ETS is a transcription factor that binds to the TERT promoter to reactivate telomerase. Li and colleagues found that p52, non-canonical NFκB subunit, forms a dimer on mutant C250T with ETS1 and ETS2 transcription factors for the activation of TERT transcription. Association of H3K4me-2 and H3K4me-3 marks and recruitment of GABP on the mutant allele occurs during the mutation of the C228T TERT promoter. GABP is an ETS family transcription factor. Its stabilization on the mutant TERT promoter, C228T leads to TERT expression which means the telomerase reactivation (Akincilar et al., 2010, Li et al., 2016).

ALTERNATIVE LENGTHENING OF TELOMERES (ALT)

Tumor cells survive the telomere shortening by ALT in which telomere length is elevated by alternative methods other than the telomerase activation. This homologous recombination-dependent maintenance of telomeres is utilized by approximately 5-15% of tumor cells. Some new studies have shown that ALT involves four steps of its mechanism:

1. DSB response
2. Homology search
3. DNA synthesis

4. Resolution

DSB signal or DSB response is generated which causes a homology search mediated by RadJ1 and HOP2-Mnd1 and the capture of the homologous sequence by the 3' end and 5' of the telomere. The propinquity of homologous sequences can be facilitated by the NR2C/F family of nuclear receptors. The mechanism of DNA synthesis and resolution of telomeres is unknown (Dilley et al., 2015).

ARTX is a chromatin-remodeling protein whose loss is associated with ALT in cancer. Rachel and colleagues showed that the regulation of the cell cycle of telomeric non-coding RNA TERRA is compromised by the loss of ARTX which leads to RPA association with telomeres after the replication of DNA. This creates a recombinogenic nucleoprotein structure. RPA recruits ATR, a recombination regulator which inhibits ALT and induces fragmentation of chromosomes and ALT cell apoptosis (Flyn et al., 2015).

TUMOR CELLS SUPPRESSION BY GENETIC MODIFICATION

Some earlier studies have shown that immortality of the cell is dominated by senescence and Roninson and colleagues have identified four dominant senescence complementation groups.

MORF4

It is one of the senescence determining genes of the complementation groups identified which has been isolated from chromosomes 4. It encodes a transcription factor-like protein and its introduction into the chromosomes of tumor cell lines that belongs to the same complementation group causes cessation of proliferation and after 18-35 replications (Roninson, 2003).

RB GENE

Senescence in tumor cells is also induced by the gene RB. RB induces senescence through a pathway which depends on the induction of some proteins:

- p27, p53, and two p53 related proteins (p63 and p73)
- Some CDK inhibitors- p21, p16, $p57^{kip2,}$ and $p15^{ink4b}$
- IGFBP-rP1- a member of IGF binding protein family

E2

E6 and E7 are the oncoproteins of PMV present in cervical carcinoma cells. Inhibition in E6 causes inhibition of p53 and inhibition of E7 inhibits RB tumor suppressor which leads to senescence of tumor cells. The E2 is a bovine PMV protein whose introduction into several cervical carcinoma cell lines of humans caused increased senescence in almost every tumor cell. E2 in association with stabilization of p53 and induction of p21 causes tumor cell suppression (Roninson, 2003).

TUMOR SUPPRESSION BY CHEMOTHERAPY

Cessation of tumor cell lines is induced by many anti-cancer agents like morphological changes and SA-β gal expression. A strongest senescent phenotype induction with DNA interacting agents (aphidicolin, doxorubicin, and cisplatin) when HT1080 fibrosarcoma cells were applied with equitoxic ID_{85} doses of different agents (Roninson, 2003). Utilization of dominant version of telomerase enzyme or antisense oligonucleotides against the telomerase RNA component inhibits the telomerase, which causes telomere shortening and cell death in tumor cell lines (Blasco, 2001).

Since the normal cell line are also affected by chemotherapy like tumor cell lines, there are various side effects caused by chemotherapy and radiation therapy. Decreased WBC count, hair-loss, nausea, and fatigues are the side effects caused by these therapies (Chen et al., 2013).

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CHINESE MEDICINE AND TELOMERE SHORTENING ROLE IN AGEING

In 1960, Leonard Hayflick found that when human cells are cultured in tissue culture, after limited cell division number they stop dividing. Alexei Olovnikov is the first scientist who connects the programmed cell division cessation which was observed by Hayflick to replication of telomeres. Alexei proposed that during the replication of DNA in cell human somatic cells might not be efficient to correct the shortening of chromosomes and for the protection of downstream gene forms this replication loss, the telomeres repeated nucleotide sequence may function as a buffer. Several studies done after the study done by Hayflick and Alexei confirmed that with each cell division there is a decrease in length of telomere in fibroblasts and somatic cells from the blood and colon with age (Aubert et al., 2008).

Many human tissues and organs, including kidney epithelial cells, lung epithelial cells, vascular endothelial cells, hepatocytes, intestinal epithelial cells, and others show shortening telomeres length. The brain of human beings is the only exception that does not show shortening of telomeres with age. There are possibilities that brain function is affected by other cell lines experiencing telomere shortening. Well, it is still unknown whether telomere shortening occurs in certain brain cell subpopulation like human neuronal stem cells. Short telomeres are observed in lymphocytes of Alzheimer disease patients compared with non-Alzheimer patients. Shortening of telomeres is also observed in the skin, which causes skin aging (Jiang et al., 2007).

CHINESE MEDICINE- ASTRAGALUS MEMBRANACEUS

Telomeres shortening can be stopped by Chinese medicine, *Astragalus membranaceus (Fisch) Bunge* (common name = Mongolian milkvetch) which improves the ability of ability of DNA repair via oxidative stress. (2,24) Wang and colleagues studies show that HDTIC-1 and HDTIC-2 can extend the lifespan of diploid fibroblasts of human fetal lungs. HDTIC-1 and HDTIC-2 are the isomers that are extracted from Mongolian milkvetch (Wang et al., 2010). Wang, P and colleagues analyzed HDTIC-1 and HDTIC-2 effects on the rate of telomeres shortening. With each cell division, the rate of telomere shortening of HDTIC-1 cultured cells were 31.5 and 41.1 of the HDTIC-2 cultured cells were observed (Wang et al., 2010).

TA-65

TA-65 is a telomerase activator which increases the length of short telomeres and hence increasing the lifespan. Bruno Bernardes and colleagues showed that TA-65 is extracted from Mongolian milkvetch roots and they are able to increase the length of telomeres and decrease the number of short telomeres and percentage of DNA damage in MEFs that are haploinsufficient and that maintain short telomeres and telomerase RNA *terc* gene, $G_3terc^{+/-}$MEF, single copy. TA-65 also elongates telomere length and prevent DNA damage in the G3terc+ / - gene (de Jesus et al., 2011).

TELOMERE SHORTENING ALSO INDUCES CARDIOMYOPATHY

Cardiomyopathy (CM) is an anatomic and pathologic examination, which is related to the dysfunction of heart muscles. CMs shows heterogeneous disease which causes ongoing heart failure. CM may be primary (mixed, genetic, or acquired) or secondary (infiltrative, inflammatory, toxic). Major types of CM are:

- Dilated Cardiomyopathy (DCM)
- Restrictive Cardiomyopathy (RCM)
- Genetic Hypertrophic Cardiomyopathy (GHCM)
- Arrhythmogenic Right Ventricular Cardiomyopathy (ARVCM)

Shortness of breath, paroxysmal nocturnal dyspnea, cough, fatigue, edema, and orthopnea are the symptoms of CM (Wexler et al., 2009).

The cause of GHCM and DCM is the mutations in proteins that have diverse function range. GCHM and DCM are defined by thickening of ventricular wall and dilation of the ventricular chamber respectively. Alex C and colleagues observed that telomeres are shorter in GHCM and DCM tissues bearing pathogenic mutations:

MYH7, DMD, TNNI3, TTN, MYBPC3, and TNNT2 of patients as compared to healthy controls. Telomeres are reduced by 26% in GHCM and 40% in DCM and this reduction is seen by performing Q-FISH (Chang et al., 2018).

β-BLOCKER

β-blocker is currently used for the treatment of GHCM. β-blocker enhances ventricular relaxation and elevates diastolic filling time. β-blocker also decreases ventricular and supra-ventricular arrhythmia susceptibility. Calcium channel blockers have negative inotropic and chronotropic effects, but not vasodilatory effect and are used either in co-occurrence with β-blocker or when β-blocker is not tolerated (Marian et al., 2010).

CONCLUSION

Telomere shortening is responsible for senescence, which occurs due to telomerase suppression and end replication problems. DSB repair pathways at telomeres ends are lethal, causing cell apoptosis and senescence by chromosome fusion. Shelterin, a six-subunit complex, which helps in maintaining telomere capping function by facilitating the formation of T-loop and D-loop. Some lifestyle factors like smoking, obesity, lack of exercise, and consumption of an unhealthy diet can cause telomere shortening. A mutation in telomerase components causes dyskeratosis cogentia (DKC), bone marrow failure, and idiopathic pulmonary fibrosis**.** A mutation in the shelterin component TINF2 causes telomere shortening. At telomeres ends, DNA damage signaling is required to be suppressed in order to maintain telomeres Cancer is a lethal disease in which tumor cells survive telomere shortening by the mean of either telomerase reactivation or alternative lengthening of telomeres. Tumor cells can be suppressed by the introduction of MORF4 gene, KB gene or by E2, a bovine PMV protein. Since, Cancer treatment by chemotherapy have adverse side-effects like hair-loss, decreased WBC count, nausea, and fatigue, telomerase inhibition at tumor cell line is the most promising treatment. Many human tissues and organs show telomere shortening with age, except the brain. Traditional Chinese medicine, *Astragalus membranaceus,* increases the lifespan of fibroblast of the human fetal lung, hence it can prevent aging. TA-65, a telomerase activator elongates the telomere end and prevent DNA damages. β-blocker cures GHCM by enhancing ventricular relaxation and elevates diastolic filling time.

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