

## **A challenging epigenetic message: telomerase activity is associated with complex changes in lifestyle**

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Short title: Telomerase and lifestyle-related epigenetic factors

Keywords: telomerase, epigenetics, cell proliferation, complex therapy, food, sport, relaxation, immune response, telomere

## Abstract

As an outcome of The 2009 Nobel Prize in Physiology or Medicine (Vogel and Pennisi, 2009) a connection has been highlighted between the length of telomeres and epigenetic effects, such as intensive changes in lifestyle and nutrition as well as behavioural and psychological factors. In this review the various elements of molecular, cell biological, nutritional and lifestyle changes are introduced and discussed.

## The genetic clock, aging, telomeres and telomerase

In humans, embryonic cells divide for about 50 times, while cells originating from an adult for fewer times, in proportion with age. This fact suggests that genetic factors, the operation of a certain genetic clock may be in the background of aging.

Telomeres are “cap-like” regions at the ends of chromosomes, which play a vitally important part in preserving the integrity and stability of chromosomes during DNA replication. Telomeres probably have a role in creating the three-dimensional structure of the nucleus and presumably in the meiotic pairing of chromosomes. Specific T- and G-rich sequences - several kilobases in length - can be found in this region. The human telomere contains a few kilobases of TTAGGG sequence. The length of the sequence may differ in different species or within the same species. Sequence length also depends on cell type, and may change with time in a given cell type.

DNA polymerase can only synthesize DNA in the 5' to 3' direction, on the other hand, it can only create a new DNA strand as the continuation of an RNA segment, the so-called primer. At the end of the synthesis, the RNA fragment is removed from the end of the new strand, and since it cannot be replaced, the 5' end of the DNA strand shortens by a fragment which equals the primer in length after each replication (Makovets and Blackburn, 2009). Further telomere loss may be caused by the fact that the primer is not necessarily created at the very end of the DNA strand. Thus, in theory, the telomeres of chromosomes become shorter and shorter depending on the number of divisions. However, when telomeres reach a certain size, the cell does not divide further, it ages. This essentially physiological process is also called replicative aging. As telomeres shorten, the expression of some tumour-suppressor genes increases in cells. One of the most important of these is *p16*, which inhibits the cyclin-dependent kinase-Cdk4/6-cyclin D- pRb-E2F pathway, and starts the aging process of the cell. Another Cdk inhibitor is *p14*, whose concentration also increases in aging cells. It binds to ubiquitin ligase (Mdm2), and in this way inhibits the degradation of p53, which increases the concentration of *p21*. The third Cdk inhibitor is *p27*, which can also be found in high concentration in aged cells. Which of the above pathways is activated as a result of telomere shortening and starts the aging processes depends partly on the cell type, but also on several, as yet unknown circumstances. At any rate, aged cells are characterized by an increased expression of tumour-suppressor genes and proteins occurring after a more or less fixed number of divisions, and this has lead researchers to the conclusion that aging is essentially a physiological process by which the organism attempts to prevent the appearance of tumours. In this respect, therefore, aging is a physiological form of tumour-suppression mechanisms.

On the basis of the above, healthy cells may only divide for a certain number of times. However, what about endlessly dividing unicellular organisms or tumour cells? In these cells and, as it turns out, in the frequently dividing embryonic cells, as well as to a certain extent in primordial germ cells, an interesting mechanism is in operation. First, the T- and G-rich 3' end of chromosomes extends beyond the other strand, curls back, and forms a so-called T-loop.

Research has shown that telomerase, which has been termed the “anti-aging” or “immortality enzyme”, makes possible the recursive division of cells without damage. According to researchers, this enzyme also plays a part in the development of the ability of cancer cells to divide endlessly.

Telomerase is synthesized by a reverse transcriptase (TERT = telomerase reverse transcriptase) enzyme (Codd et al. 2010), This is a ribonucleoprotein; a short (11

nucleotides), AC-rich segment of its RNA component (TERC = telomerase RNA component; 451 nucleotides in humans) serves as a template complementary to the end of the chromosome, attaching to it and elongating it with hexameric repeat sequences TTAGGG (at which time the T-loop is undone). The RNA component of the enzyme can be found in all mammalian cells, but the protein subunit (TERT) is only expressed in early embryonic cells and germline cells. Telomerase ensures, at least in certain cells, that chromosomal DNA is not shortened during replication.

On the basis of the above we would expect the telomeres of the chromosomes of tumour cells to be of the same length as those of the embryonic cells. However, research has shown that the telomeres of tumour cells may be significantly shorter than the telomeres of somatic cells. A possible explanation for this is that in the developing embryo, or in the primordial germ cell telomeres do not shorten, as a result of the activity of telomerase.

Following organ development however, telomerase is switched off, repressed in somatic cells, thus the telomeres of the cells become shorter and shorter from division to division by time. After reaching a certain

“shortness”, normal division of cells is not possible any longer, the genetic material is more and more damaged, cells become distorted, age and die. Meanwhile if telomerase is activated, cells do not lose their telomeres despite repeated division (Figure 1.); they become immortal.

Since telomerase does not function in most somatic cells, but is indispensable to tumour cells, it may be an important and possibly specific target of the development of antitumor medication. This, among others, is why the operation of the enzyme telomerase is the centre of attention. However, these studies are only in an initial stage. It is very probable that the premature death of the first cloned mammal, the lamb named Dolly, is also related to telomeres and their “congenital” shortness (Niemann et al.). In this case cloning was performed by removing the nucleus of a sheep egg and replaced it with the nucleus taken from a somatic cell of an adult animal (Dolly’s mother), and the pregnant animal carried this “test tube baby” (Wilmut et al.). Of course, the DNA of the somatic cell nucleus contained all genetic information, but the telomeres of the chromosomes were already shorter than, the telomeres of an embryonic cell.

Dolly lived only for six years, whereas the average life expectancy in sheep is 10-12 years. It is interesting, but not surprising in the light of the above, that the animal showed signs of aging much earlier than expected, it suffered from chronic polyarthritis and lung disease, which otherwise are old-age diseases of sheep. This suggests some relation between telomere length and natural as well as pathological aging (Shiels et al.).

### **Therapeutic dietetic details and metabolic consequences of a known lifestyle modification program**

*Lancet Oncology* published an interesting article in November 2008 on metabolic consequences of lifestyle changes (Ornish, et al. 2008) Authors proved that the progression of severe coronary artery disease may be arrested by comprehensive epigenetic factors. Some lab readings may even be reversed to a certain extent without the use of drugs or surgical intervention, as it has been published earlier (Ornish et al, 1990).

### **The outlines of Ornish therapy are based on four components:**

1. A low-fat, almost lacto-ovo vegetarian diet, whose basic principle is the elimination of cholesterol and saturated fats. Fats may represent 10% of the total calorie intake, while the upper limit of daily cholesterol intake is 5 mg. Besides these, a further 20% of the proposed total energy should come from proteins and 70% from carbohydrates. This diet is very similar to the US-Recommended Daily Allowances.

2. A gradually constructed regular exercise program consisting of aerobic exercise and a daily yoga practice.
3. Stress management - achieved through yoga, meditation, special breathing exercises and relaxation.
4. Participation in a support group on a weekly basis, whose atmosphere can help patients open up and talk about their deeper thoughts and emotions and experience the supportive strength of the group.

The number of anginas was decreased as soon as two weeks in a group of patients waiting for cardiovascular or bypass surgery. After one year of Ornish therapy, images showed significantly fewer – by 82% – cases of atherosclerosis, and angina symptoms decreased by 91% (Gould et al, 1992, 1995, Ornish, 1998, Ornish et al, 1998).

In 2008, the *American Journal of Cardiology* published the results of a multisite trial examining the effect of the Ornish lifestyle program on angina pectoris and atherosclerotic risk factors (Frattaroli et al., 2008). During the lifestyle intervention program 757 men and 395 women with stable angina from coronary sclerosis were observed at 22 trial sites. The average age was 62 years. Diet modification was done on the basis of the Ornish principles (10% of calories from fats, plant-based diet). With moderate physical activity (3 hours/week) and stress relief (1 hour daily), 74% of the patients became angina-free in 12 weeks, and another 9% experienced milder angina complaints.

Aldana et al.(2007) launched a randomized trial in 2007 with the participation of 93 clinically proven coronary patients, in which 47 patients received traditional cardiology rehabilitation, and 46 patients followed the Ornish program. Based on the values of carotid artery ultrasound examination and other risk factors measured at the start, at 6, and at 12 months, it was observed that there was no significant reduction as regards the carotid intima-media thickness between the Ornish and the rehabilitation group. However at the same time, the number of patients suffering from angina symptoms decreased by 44% in the Ornish group, and by 12% in the control group (Aldana et al., 2007).

During the trials, beyond the improvement in clinical condition, a significant decrease in cardiovascular risk factors, especially metabolic parameters [LDL-cholesterol, body mass index (BMI), waist circumference, systolic and diastolic blood pressure] was also observed (Ornish, 1998, Ornish et al, 1998, Frattaroli et al., 2008).

All those information indicates some correlation between epigenetic factors and clinical progress of cardiovascular pathology.

Further randomized and controlled trials by Ornish et al. (2005) suggest that the conscious modifications in environmental factors mentioned above resulting in epigenetic alterations can also inhibit the progression of prostate cancer in certain circumstances (Ornish et al., 2005).

An other publication (Ornish et al., 2008) presents clinical outcome of non-smoking patients with histologically proven prostate tumours. The trial was conducted on ninety-three volunteers divided into two groups based on individual choice and preset recruitment criteria (PSA 4- 10/ng/ml, Gleason <7), in a so-called “watchful waiting” program according to the protocol (pT0, N0, M0, G1 stages) (Ornish’s terminology, “active surveillance” is even more expressive).

In the lifestyle modification group (nearly vegetarian diet, 6x30 minutes’ walking weekly, stress relief, yoga, breathing exercises, imagining, progressive relaxation, support group meeting once weekly), there was a significant difference in the progression of the disease (by PSA- monitoring) compared to the control group, where the nutritional and other programs were not required, but patients could freely choose from among the possibilities. It was an even more impressive finding, that the sera of the experimental lifestyle modification group patients in vitro inhibited the growth of prostate cancer cells (LNCaP)

by 70%, while that of the control group only by 9%.

After one year, no oncological treatment was initiated in any of the 41 patients of the experimental group, while treatment was started in six patients of the control group (radical prostatectomy, radiation therapy, androgen deprivation). The difference was significant.

Quality of life (QoL), improved in the experimental group, characterized by sexual functions, stress tolerance ability, less anxiety and feelings of weakness.

After two years 13 patients from the control group and 2 patients from the experimental group received conventional oncological treatment (Frattaroli et al., 2008), and after four years this number increased to 21 in the control group and 6 in the experimental group.

### **The effect of conscious epigenetic modification by lifestyle changes on telomerase activity in peripheral mononuclear cells**

The peripheral mononuclear cells telomerase activity followed before and after three months of intensive and strictly controlled complex lifestyle changes of prostate cancer patients (Ornish et al., 2008).

The authors observed significant telomerase enzyme activity increase (by almost 30%!) in the peripheral mononuclear cells (PBMC) following intensive lifestyle changes after three months and at the same time LDL (low-density lipoprotein) and cholesterol levels decreased. This might suggest that decreased telomerase activity in the cells of the immune system telomere shortening, are among the risk factors, leading to the weakening of the immune response in malignant and cardiovascular diseases. This may well be in connection with the fact that stress hormones (catecholamines, cortisol) induce oxidative stress, which also exerts a telomerase inhibiting effect on peripheral lymphocytes. Oxidized LDL similarly inhibits telomerase activity.

Since PSA concentration remained stable in the patients receiving lifestyle therapy, but free PSA levels were significantly reduced, one suggests, that telomerase activity in PBMC cells are not directly related to the progression of prostate cancer. No infections occurred, a decrease in CRP levels and no sign of infection, impaired immune protection was observed among participating patients.

According to the opinion of the authors, telomerase activity levels yield a more direct and potentially earlier prediction than telomere length. The rate of shortening is 30-60 nucleotide bases per year, therefore, the three months observation period obviously could not detect significant changes. One speculates, that the increase in telomerase activity and the protective effect of the immune system may be connected at the level of long-term cell viability and/or genome stability (e.g. DNA repair).

It is generally accepted that oxidative stress and chronic inflammation are associated with risk factors like unhealthy diet and insufficient exercise. The connection between morbid obesity, metabolic syndrome and chronic emotional tension was demonstrated by the Harvard Nurse's Health Study.

Increased consumption of red and processed meats, sweets, desserts, fries, and refined grains enhanced inflammation parameters, contrary to increased consumption of fruit, vegetables, ocean fish, poultry and unrefined grains (whole grains) (Liu et al., 1999).

It has also been shown (Frattaroli et al., 2008) that telomere shortening may be associated with obesity, and this process may be influenced or reversed by radical changes.

We believe, this is one of the first prospective longitudinal study which links complex and self-imposed environmental, likely epigenetic changes to increased telomerase enzyme activity. However, since several mutations of the genes encoding the telomerase enzyme activity are known, the importance of genetic factors has to be considered as well.

### **What can be the link between behavioural and psychological factors and telomere shortening?**

The biological effects of stress was clearly proven by a Hungarian scientist, *János Selye*. Epel et al.,(2004) checked telomerase activity and telomere shortening in mothers of chronically ill and healthy children to compare how stress gets “under the skin”. In all other aspects, the mothers were so-called social twins of each other, however, one of the groups lived in a severe stress situation for long years, since caring for the sick child and the difficulty of coping with this situation meant a continuous, severe additional adaptive burden. The study found that the mothers of sick children showed significantly lower telomerase activity and significant telomere shortening, which corresponded to a cellular aging of 9-17 years compared to the mothers of healthy children. The extent of cellular aging showed great deviation within the group and was clearly associated with the level of stress. Those mothers who perceived their situation as more stressful aged much faster than those who could accept this difficult life situation (Epel et al., 2004).

Significant telomere shortening was also observed among caregivers of Alzheimer’s disease patients compared to the control group matched for age and gender. In caregivers of Alzheimer’s disease patients – telomere shortening showed a close connection with depression symptoms (Damjanovic et al., 2007). Based on these trials, telomere shortening seems to serve as a biological marker of experiencing a chronic stress condition, which can be regarded as a very convincing model of psychobiological interaction. Several recent studies have emphasized the parallelism between chronic stress and depression. Childhood and family background, the coping ability, social competences and life events of the individual play a decisive part in the development of depressive symptoms. Since self-esteem depends on what goals people set for themselves and when they feel successful, attitudes, goals and values have a fundamental role in preventing depression. A great number of follow-up trials proved the independent risk represented by depression especially in cardiovascular diseases, but also as regards to total mortality (Kopp and Rethelyi, 2004).

The adverse health effect of chronic stress are indirectly transmitted by such inadapative behavioural patterns as smoking, excessive alcohol consumption and dysfunctional eating habits, which relieve tension in the short term, but have an adverse health effect in the long term.

According to a Hungarian follow-up trial concerning the efficiency of cardiac patients rehabilitation by the Williams LifeSkills program (Antoni et al., 2002, Williams and Williams, 1997, Bishop et al., 2005), stress-related somatic and psychological symptoms decreased in the participants of the program .

Analyzing the possible causes and molecular genetic aspects of premature health deterioration and death is especially important in Hungary, where according to the 2009 OECD summary the mortality rate of middle aged men is 15% (!) higher than in all Western European countries. The premature mortality rate in men is worse only in Ukraine, Russia and the Baltic countries. This so-called “Central and Eastern European health paradox” (Stauder et al., 2010, Weidner et al., 2000) can be regarded as a natural experimental situation for examining the effects of chronic stress on health and open possible aspects of primer prevention.

Modern medical science in general was sceptic as regards the role of lifestyle modification, however, the above mentioned group of information open new perspectives and definitely worthy of further thought and consideration.

Now it is obvious that certain factors affected by environmental/epigenetic conditions which are proven to be associated with an increase in the risk of tumour formation and cardiovascular diseases, have a significant but apparently reversible effect on the “maintenance system” of telomeres. The cited publications of *Ornish* and *Blackburn* regarding the telomerase activity changes induced by epigenetic factors are impressive. It is evident that further appropriately randomized studies with a greater number of cases are necessary for drawing significant conclusions, but the promising nature of the available results is obvious.

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## Legends to Figures

### Figure 1.

Short or unprotected ("accessible") telomeres cause genome instability, which leads to a stop in the proliferation and to the death of the cell (left side of the figure). Occasionally, genetic instability results in the transformation of the cell to cancer (right side of the figure). Increased telomerase activity in the cells may be associated with increased immune response.

