Cell Biology International

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

András Falus*, István Marton[1][†], Erika Borbényi[†,‡], Ádám Tahy[†], Pál Karádi[†], János Aradi[§], Adrienne Stauder[‖] and Mária Kopp[‖]

* Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary
[†] Quintess Health Centre, Budapest, Hungary
[‡] Radiology and Oncotherapy Clinic, Oncology Care Institute, Semmelweis University, Budapest, Hungary
[§] Institute of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[‖] Department of Behavioural Sciences, Semmelweis University, Budapest, Hungary

Abstract

As an outcome of The 2009 Nobel Prize in Physiology or Medicine, 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.

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

1. The genetic clock, aging, telomeres and telomerase

In humans, embryonic cells divide approx. 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′→3′ direction, and 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 that 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 Cdk4/6 (cyclin-dependent kinase-4/6)–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 (murine double minute 2)] 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 and 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 led 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 mechanism.

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 extend beyond the other strand, curls back and forms a so-called T-loop.

Research has shown that telomerase, which has been termed as 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.
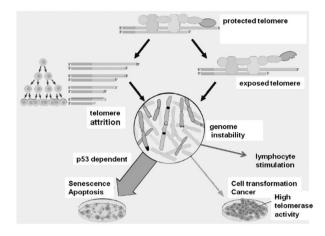
Figure 1   The role of telomeres
Short or unprotected ('accessible') telomeres cause genome instability, which leads to a stop in the proliferation and to the death of the cell (left side of the Figure). Occasionally, genetic instability results in the transformation of the cell to cancer (right side of the Figure). Increased telomerase activity in the cells may be associated with increased immune response.

Telomerase is synthesized by a reverse transcriptase [TERT (telomerase reverse transcriptase)] enzyme (Codd et al., 2010), This is a ribonucleoprotein; a short (11 nt), AC-rich segment of its RNA component [TERC (telomerase RNA component); 451 nt 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 germ-line 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, and thus, in time, the telomeres of the cells become shorter and shorter from division to division. After reaching a certain 'shortness', normal division of cells is no longer possible, the genetic material is further damaged, cells become distorted, age and die. Meanwhile, if telomerase is activated, cells do not lose their telomeres, despite repeated division (Figure 1), and 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 anti-tumour medication. This, among other reasons, 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., 2008). 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., 1997). 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 6 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., 1999).

2. 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., 2008a). 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 has been published previously (Ornish et al., 1990).

3. Ornish therapy

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 Allowance.
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 strength of the group.

The number of anginas was decreased as soon as 2 weeks in a group of patients waiting for cardiovascular or bypass surgery. After 1 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., 2008a). 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 h/week) and stress relief (1 h 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 (low-density lipoprotein)-cholesterol, BMI (body mass index), waist circumference, systolic and diastolic blood pressure] was also observed (Ornish, 1998; Ornish et al., 1998; Frattaroli et al., 2008a).

All this 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).

Another publication (Ornish et al., 2008b) presented clinical outcome of non-smoking patients with histologically proven prostate tumours. The trial was conducted on 93 volunteers divided into two groups based on individual choice and preset recruitment criteria [PSA (*Pisum sativum* agglutinin) 4–10 ng/ml, Gleason <7], in a so-called 'watchful waiting' program according to the protocol (pT0, N0, M0 and G1 stages) (Ornish's terminology, 'active surveillance', is even more expressive).

In the lifestyle modification group (nearly vegetarian diet, 6×30 min walking weekly, stress relief, yoga, breathing exercises, imagining, progressive relaxation and support group meeting once weekly), there was a significant difference in the progression of the disease (by PSA monitoring) compared with 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 1 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 and androgen deprivation). The difference was significant. QoL (quality of life) improved in the experimental group, characterized by sexual functions, stress tolerance ability, less anxiety and feelings of weakness.

After 2 years, 13 patients from the control group and 2 patients from the experimental group received conventional oncological treatment (Frattaroli et al., 2008b), and after 4 years this number increased to 21 in the control group and 6 in the experimental group.

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

Peripheral mononuclear cell telomerase activity was followed before and after 3 months of intensive and strictly controlled complex lifestyle changes of prostate cancer patients (Ornish et al., 2008b). The authors observed significant telomerase enzyme activity increase (by almost 30%!) in PBMC (peripheral blood mononuclear cell) following intensive lifestyle changes after 3 months and at the same time LDL and cholesterol levels decreased. This might suggest that decreased telomerase activity in the cells of the immune system causing telomere shortening is, among the risk factors, leading to the weakening of the immune response in malignant and CVDs (cardiovascular diseases). This may well be in connection with the fact that stress hormones (catecholamines and cortisol) induce oxidative stress, which also exert 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 PBMCs are not directly related to the progression of prostate cancer. No infections occurred, a decrease in CRP (C-reactive protein) 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 nt bases per year; therefore the 3 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 Nurses' Health Study.

Increased consumption of red and processed meats, sweets, desserts, fries and refined grains enhanced inflammation parameters, in contrast 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., 2008a, 2008b) that telomere shortening may be associated with obesity, and this process may be influenced or reversed by radical changes.

We believe that this is one of the first prospective longitudinal studies, 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.

5. What can be the link between behavioural and psychological factors and telomere shortening?

The biological effects of stress were 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 with 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 AD (Alzheimer's disease) patients compared with the control group matched for age and gender. In caregivers of AD 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 CVDs, and also with regard to total mortality (Kopp and Rethelyi, 2004).

The adverse health effects of chronic stress are indirectly transmitted by such inadaptive behavioural patterns such 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 (Williams and Williams, 1997; Antoni et al., 2002; Bishop et al., 2005), stress-related somatic and psychological symptoms decreased in the participants of the program.

Analysing the possible causes and molecular genetic aspects of premature health deterioration and death is especially important in Hungary, where, according to the 2009 Organization for Economic Co-operation and Development summary, the mortality rate of middle-aged men is 15% higher than in all other 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 is sceptical as regards the role of lifestyle modification; however, the above-mentioned information opens new perspectives and is 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 CVDs, 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.

References

Aldana SG, Greenlaw R, Salberg A, Merrill RM, Hager R, Jorgensen RB. The effects of an intensive lifestyle modification program on carotid artery intima-media thickness: a randomized trial. Am J Health Promot 2007;21:510–6.

Antoni MH, Cruess DG, Klimas N, Maher K, Cruess S, Kumar M et al. Stress management and immune system reconstitution in symptomatic HIV-infected gay men over time: effects on transitional naive T cells (CD4+CD45RA+CD29+). Am J Psychiatry 2002;159:143–5.

Bishop GD, Kaur M, Tan VL, Chua YL, Liew SM, Mak KH. Effects of a psychosocial skills training workshop on psychophysiological and psychosocial risk in patients undergoing coronary artery bypass grafting. Am Heart J 2005;150:602–9.

Codd V, Mangino M, van der Harst P, Braund PS, Kaiser M, Beveridge AJ et al. Common variants near TERC are associated with mean telomere length. Nat Genet 2010;42:197–9.

Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H Laskowski B et al. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. J Immunol 2007;179:4249–54.

Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD et al. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci USA 2004;101:17312–5.

Frattaroli J, Weidner G, Merritt-Worden TA, Frenda S, Ornish D. Angina pectoris and atherosclerotic risk factors in the multisite cardiac lifestyle intervention program. Am J Cardiol 2008a;101:911–8.

Frattaroli J, Weidner G, Dnistrian AM, Kemp C, Daubenmier JJ, Marlin RO et al. Clinical events in prostate cancer lifestyle trial: results from two years of follow-up. Urology 2008b;72:1319–23.

Gould KL, Ornish D, Kirkeeide R, Brown S, Stuart Y, Buchi M et al. Improved stenosis geometry by quantitative coronary arteriography after vigorous risk factor modification. Am J Cardiol 1992;69:845–53.

Gould KL, Ornish D, Scherwitz L, Brown S, Edens RP, Hess MJ et al. Changes in myocardial perfusion abnormalities by positron emission tomography after long-term, intense risk factor modification. JAMA, J. Am. Med. Assoc. 1995;274:894–901.

Kopp MS, Réthelyi J. Where psychology meets physiology: chronic stress and premature mortality – the Central–Eastern European health paradox. Brain Res Bull 2004;62:351–67.

Liu S, Stumpfer M, Hu F, Giovannucci E, Rimm E, Manson JE et al. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr 1999;70:412–9.

Makovets S, Blackburn EH. DNA damage signalling prevents deleterious telomere addition at DNA breaks. Nat Cell Biol 2009;11:1383–6.

Niemann H, Tian XC, King WA, Lee RS. Epigenetic reprogramming in embryonic and foetal development upon somatic cell nuclear transfer cloning. Reproduction 2008;135:151–63.

Ornish D. Avoiding revascularization with lifestyle changes: The Multicenter Lifestyle Demonstration Project. Am J Cardiol 1998;82:72T–76T.

Ornish D, Brown SE, Scherwitz LW, Billings JH, Armstrong WT, Ports TA et al. Can lifestyle changes reverse coronary atherosclerosis? The Lifestyle Heart Trial. Lancet 1990;336:129–33.

Ornish D, Scherwitz LW, Billings JH, Brown SE, Gould KL, Merritt TA et al. Intensive lifestyle changes for reversal of coronary heart disease. JAMA, J. Am. Med. Assoc. 1998;280:2001–7.

Ornish D, Weinder G, Fair WR, Marlin R, Pettengill EB, Raisin CJ et al. Intensive lifestyle changes may affect the progression of prostate cancer. J Urol 2005;174:1065–9.

Ornish D, Lin J, Daubenmier J, Weidner G, Epel E, Kemp C et al. Increased telomerase activity and comprehensive lifestyle changes: a pilot study. Lancet Oncol 2008a;9:1048–57.

Ornish D, Magbanua MJ, Weidner G, Weinberg V, Kemp C, Green C et al. Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention. Proc Natl Acad Sci USA 2008b;105:8369–74.

Shiels PG, Kind AJ, Campbell KH, Waddington D, Wilmut I, Colman A, Schnieke AE. Analysis of telomere lengths in cloned sheep. Nature 1999;399:316–7.

Stauder A, Konkoly Thege B, Kovács ME, Balog P, Williams VP, Williams RB et al. Worldwide stress: different problems, similar solutions? Cultural adaptation and evaluation of a standardized stress management program in Hungary. Int J Behav Med 2010;17:25–32.

Weidner G, Kopp MS, Kristensen M (eds). Heart disease: environment, stress and gender. NATO Science Series, Life and Behavioural Sciences IOS Press; 2000.

Williams VP, Williams RB. Life-skills eight simple ways to build stronger relationships, communicate more clearly and improve your health. New York: Times Books/Random House; 1997.

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997;385:810–3.