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The Importance of the Hayflick Limit, Telomeres and Telomerase in Aging And Development of Pathology
(Review)

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Abstract:
The Hayflick limit, telomeres and telomerase are the subject of many studies and researchers are looking for ways to modulate their function in order to treat cancer, slow aging and increase longevity. They play a critical role in cellular aging and the development of pathologies. Understanding the mechanisms underlying these processes can provide insight into diseases associated with aging and potential interventions to slow aging and improve overall health.

Keywords: Hayflick limit, telomeres, telomerase, telomere theory of aging, telomere length.

Introduction

The Hayflick limit is a theoretical concept that deals with the limited ability of cells to divide, known as the "lifespan of a cell". It was put forward by the American scientist Leonard Hayflick in 1961 and explains that during life, somatic (not germinal) cells of the human body can divide only a certain number of times, which usually ranges from 40 to 60 (50±10) times in culture [19; 15]

Hayflick proved that the age of cells depends not so much on the length of time, but on the number of DNA replication cycles. Tissue culture cells subjected to cryogenic freezing could still undergo the same number of divisions after thawing [20]. Australian Nobel laureate Frank McFarlane Burnet called this phenomenon the “Hayflick limit” and its existence proved once and for all that the cessation of cell division depends on some internal factors [35].

Hayflick's work refuted the opinion that had existed in science since the beginning of the 20th century thanks to the experiments of Alexis Carrel. Although August Weismann put forward the hypothesis back in 1889 that the number of cell divisions is limited, Carrel’s experiment with the chicken heart forced scientists to forget about it.

Further research showed that Carrel's experiment was falsified, and he himself most likely knew about it [16]. Each time he added nutrients to the medium, new embryonic cells were added along with them. The supposedly eternal chicken tissue was constantly renewed by these new cells, and after just a few months it no longer contained any of the cells with which Carrel began his experiment. But now that the "Hayflick limit" has gained universal acceptance, researchers are faced with the question of why this limit exists at all. The answer to this could indicate the cause of aging of cells, and, consequently, of people.

Regular human cells contain twenty-three pairs of chromosomes; male and female reproductive cells contain one copy of each of the twenty-three chromosomes, forming a complete set upon fertilization. After Hayflick's discovery, research began to study the mechanisms underlying cellular aging.From the very first works devoted to this problem, the main attention was focused on the very tips of chromosomes.

The scientists noticed that while the central regions of the chromosomes contained unique DNA sequences that were similar for all cells within a species and were vital for directing the synthesis of the cell's essential components, the sequences at the ends of the chromosomes were very different. Firstly, during replication, the cell could not create a complete copy of the terminal sections of its DNA [41]. And secondly, the length of these regions varied in different cells, which was unusual, given the high degree of constancy of the genome structure.

In 1932, geneticists Barbara McClintock and Herman Möller independently established that fragmentation of chromosomes and the appearance of additional ends lead to chromosomal rearrangements and chromosome degradation. Only the regions of chromosomes adjacent to their natural ends remain intact. Deprived of terminal telomeres, chromosomes begin to fuse with greater frequency, which leads to the formation of severe genetic abnormalities. Scientists have come to the conclusion that the natural ends of chromosomes are protected by special structures, which G. Möller proposed to call telomeres (from the Greek "telos" - end and "meros" - part). Since that time, many scientists have been studying the role of telomeres and the telomerase enzyme in the cell cycle, which has allowed them to accumulate a considerable amount of knowledge on this issue.

Telomeres are the ends of chromosomes that protect them from damage and loss of information during cell division. They consist of repeated nucleotide sequences, such as TTAGGG in most higher organisms. The limitation in the number of cell divisions is often explained by shortening of telomeres

With age, telomeres shorten with each cell division, and upon reaching a critical length, the cell stops dividing and goes into a state of apoptosis (programmed cell death) or into a state of aging [18; 35].

Hayflick's theory has had a significant impact on various fields of biology and medicine, allowing for a better understanding of the processes of organism development and the formation of various types of cells. This theory has practical applications, including

studying the development of embryonic stem cells and work in the field of regenerative medicine

Telomere theory of aging

The telomere theory of aging was proposed in 1971 by the Soviet biochemist and geneticist Alexei Olovnikov [4; 33]. This theory suggests that the aging process is associated with the shortening of telomeres - the protective caps at the ends of chromosomes. As telomeres shorten with each cell division, cells eventually reach a point where they can no longer divide, resulting in decreased function of tissues and organs. According to this theory, when telomeres become too short, the cell stops dividing and eventually dies.Thus, the level of telomeres in cells can be used to determine their age. He also suggested that there must be a special biological mechanism that prevents this effect. It was also assumed that the named mechanism operates in reproductive cells, as well as in cancer cells and in the cells of organisms that reproduce vegetatively, and does not operate in most other cases, in particular, in many of our somatic cells. Subsequently, the enzyme predicted by Olovnikov, which compensates for DNA shortening, was actually found in all types of cells listed above and was named telomerase. The enzyme telomerase is responsible for extending a repeatedly repeated hexanucleotide (TTAGGG in humans) at the ends of nuclear DNA, forming a telomere. As a result, the shortening of linear DNA affects only this non-transcribed text of the telomeric region of the chromosome, but does not lead to the loss of hereditary information and does not disrupt the mechanism for reading it. At a certain stage of development, which occurs during early embryogenesis, the gene encoding telomerase is switched off in the vast majority of human somatic cells. Thus, the genome becomes defenseless against the danger of shortening. Slowly but surely, the telomere shortens, which leads to deterioration in the functioning of chromosomes. This deterioration occurs long before the entire telomere disappears and the degradation of the sense DNA regions begins.The fact is that the telomere, in addition to protecting against the loss of genetic material during replication, also plays some still unclear structural role in the arrangement of chromosomes inside the nucleus and in their proper functioning.

Olovnikov's telomere theory has received wide recognition and research in this area continues to this day. It inspired the creation of research methods and possible approaches to prolonging life and combating aging.

Modern researchers continue to study the telomere theory of aging. Among the famous scientists who were the first to use the telomere theory in their research was Elizabeth Blackburn. In 1978, she published the first data she obtained at Yale University on the terminal regions of the chromosomes of the simplest single-celled organisms [9]. E. Blackburn discovered something very interesting: unlike the rest of the chromosomes, which consist of random nucleotide sequences that can control protein synthesis and perform other important functions, the terminal regions are repeats of the same

sequences, which are the same across a wide variety of species and species. do not have any specific encoded content. The number of such repeats varies from cell to cell [17], and the same is observed in the cells of the human body [32].

Further studies showed that not only does the length of these terminal sections, called telomeres, vary from cell to cell, but more importantly, they shorten with each new division [18]. These observations gave serious reasons to believe that it is telomeres that are responsible for the existence of the Hayflick limit, since at the moment when they are completely shortened, the state of the cell becomes unstable and the process of apoptosis starts.

Telomerase

In 1985, Blackburn and one of her students named Carol Greider discovered telomerase, an enzyme that is responsible for the synthesis and elongation of telomeres [26]. E. Blackburn and K. Greider conducted a study to find out whether some hitherto unknown enzyme was involved in the formation of DNA telomeres. On Christmas Day 1984, K. Greider discovered signs of enzymatic activity in a cell extract. The discovered enzyme was named telomerase by E. Blackburn and K. Greider. After isolating and purifying it, scientists determined that it consists not only of protein, but also of RNA, which contains the same sequence as the telomere. Thus, RNA serves as a template for building the telomere, while the protein component of the enzyme is required directly for enzymatic activity. Telomerase extends the DNA of the telomere, providing a platform that in turn allows DNA polymerases to copy the entire length of the chromosome without loss of genetic information. Thus, the chromosome is not shortened during copying.

Telomerase is an RNA-dependent DNA polymerase (or reverse transcriptase). The main purpose of this enzyme is to synthesize tandemly repeating segments of DNA that make up the telomeric DNA chain.In ordinary (somatic) cells, of which the body mainly consists, telomerase “does not work”, so telomeres are shortened with each cell division, which ultimately leads to a state of “arrest” (cell senescence - aging, decrepitude of the cell), when a cell is no longer able to divide.

In embryonic and pluripotent stem cells, telomerase expression maintains a constant level of telomere length. Telomerase is significantly expressed in these cells. In addition, elevated levels of telomerase are found in hematopoietic stem cells, activated T lymphocytes, and most human tumors. [eleven].

Elizabeth Blackburn, along with Jack Szostak and Carol Greider, was awarded the Nobel Prize in Physiology or Medicine in 2009 for their discovery of how chromosomes are protected by telomeres and the enzyme telomerase.

Regulated repair of chromosomal DNA is necessary to compensate for shortening that occurs as a result of nuclease activity and incomplete end DNA replication. The multicomponent system of “telomere homeostasis” prevents, on the one hand, excessive elongation of telomeres, and on the other hand, critical shortening, which can lead to

DNA damage and genome instability. Despite the participation of telomerase in the processes of restoration of telomere length in each cell cycle, restoration in somatic cells, unlike embryonic stem cells, is not complete, and most somatic cells lose telomere sections with each replication cycle.In vascular endothelial cells, smooth muscle cells and cardiomyocytes, telomerase activity is insufficient. Artificially induced expression of the protein component of telomerase in the culture of these cells leads to restoration of telomerase activity and stopping the aging process. Scientists have noted that the shorter the telomeres, the older the cell. And vice versa: if the activity of telomerase, which completes telomeres, is high and the same telomere length is constantly maintained, the cell does not age. The presence of age-associated diseases, such as heart failure, arterial hypertension (AH), and atherosclerosis predetermined the detection of shorter telomeres, but it has not yet been possible to establish what is the cause and what is the effect. Based on these findings, it has been suggested that telomere length may be a biomarker of aging and age-related diseases. It was also found that disruption of the functioning of telomerase is sufficient for the development of severe damage to telomeres, which is not accompanied by their shortening, but leads to premature tissue degeneration, the formation of foci of neoplasia and cell death. Since telomere degradation affects the aging process of the heart, major research is currently aimed at preserving the integrity of DNA and especially its telomeric regions. Thus, the telomere theory has become the subject of many modern studies, the practical implementation of ideas about slowing down the aging process and a number of diseases. However, further research is required to resolve many unclear issues.

Telomere length decreases with age. The most intense reduction in telomere length in humans occurs in the first years of life (due to the body's needs for growth) and after 60 years (due to disturbances in the telomere restoration mechanism) [28;38]. B lymphocytes are an exception to this rule. The lengthening of telomeres in the B-cell line of lymphocytes reflects the need for extensive division of these cells (due to the peculiarities of their functions - clonal secretion, as well as the synthesis and production of antibodies). Telomerase activation is triggered by a feedback principle in response to shortening telomere length. However, this mechanism has a limit in all cells; over time, there is a decrease in telomerase activity and, as a consequence, a decrease in the average telomere length, as well as a decrease in the possibility of its restoration. Thus, a barrier is created for tumor degeneration of the cell [28].

By adding additional repeats, telomerase can increase the length of telomeres in cells. Experiments by scientists have shown that introducing the telomerase gene into otherwise normal cells can significantly increase their lifespan [14].Moreover, it has become known that re-activation of telomerase in mice that have aged prematurely due to the fact that the work of telomerase in their cells was initially suppressed leads to the disappearance of signs of old age [27]. Nowadays, the terminal regions, which first attracted the attention of scientists back in the 1930s when it was noticed that they do

not participate in the exchange of genetic material between chromosomes, are considered key to maintaining the balance between life and death of cells.

Telomeres, like tree rings, provide a very clear picture of an individual's continuous struggle for survival. When they become very short, cells can no longer divide without losing important genetic material. The result is an unstable condition that creates the conditions for cell damage and eventual death. Disturbances in DNA structure are a characteristic feature of the aging process of cells, and in addition to telomere shortening, there are several other mechanisms that contribute to this. Damage to mitochondria (the cellular powerhouse) leads to the release of toxic substances that can hasten the onset of apoptosis.

It is now also known that a moderate and balanced diet promotes long life [30]. Research has shown that lifestyle factors such as diet, exercise and stress can influence telomere length and telomerase activity. For example, healthy lifestyle habits such as regular exercise and a balanced diet are associated with longer telomeres and higher telomerase activity.Growth hormone and insulin-like growth factor IGF1, which are responsible for growth in humans and many other organisms, decrease in activity as we age. However, reducing food intake by 20–40% purposefully suppresses their synthesis and puts the body into survival mode. By noticing that the supply of nutrients is limited, the cell slows down the processes of growth, metabolism and division, thus reducing the likelihood of errors occurring. This leads to an increase in life expectancy. In addition, as we age, our body begins to suffer from a reduction in the number and quality of stem cells, which could otherwise ensure the constant renewal of various tissues.

Cellular aging appears to be as carefully regulated as any other aspect of cellular life. From this it is obvious that old age is achieved, and does not just happen. The reason why cells age and are then replaced by new ones is, as always in the microscopic cellular world, the need to continue life. Although cells, like ourselves, fight aging with powerful troubleshooting mechanisms, they also have the ability to detect when the level of accumulated damage has reached a critical level. It is at this moment that tissues get rid of those cells that have already aged, in order to protect the entire organism from uncontrolled death and necrosis. The period from critical telomere shortening to cell death can last for several months or even years. The cell loses the ability to divide, but can maintain metabolic activity. Activation of telomerase in a somatic cell with a critical telomere length leads to tumor transformation of this cell.

While telomerase, which allows cells to divide indefinitely, may seem like the true philosopher's stone of our time, its work has dark and controversial implications. Serving not as a source of life, but as a harbinger of death, it is involved in the occurrence of almost all types of cancer [29]. Cancer cells use telomerase to gain the ability to continuously divide: they constantly increase the length of the terminal sections of their chromosomes, delaying death and endlessly multiplying their number (i.e., acquiring immortality - immortalization)

In the context of oncology, disruption or bypass of the Hayflick limit is a key mechanism in cancer development. Cancer cells often bypass this limit by activating telomere maintenance mechanisms such as telomerase, allowing them to divide uncontrollably and form tumors. Thus, the Hayflick limit plays an important role in maintaining the integrity of genetic material and preventing unrestricted cell growth, which is key in the development of cancer.

At the cellular level, immortality already has both a name and a face: it is called cancer and does not look particularly attractive.The paradox of telomerase, that this enzyme is necessary both for prolonging the life of the body and for the proliferation of cancer cells, is repeated in connection with many other attempts to avoid cell death. Our attempts to increase human lifespan also resemble what happens at the cellular level, and lead to changes in ecology and the circumstances of modern death. Our ongoing struggle with aging, disease and death is causing profound social and economic changes.

Research has shown that telomerase, the enzyme responsible for maintaining telomeres, is elevated in most cancer cells, allowing them to continually divide and avoid cell death. This has led to interest in targeting telomerase as a potential cancer treatment.

Recent studies are exploring the role of telomeres and telomerase in aging and stem cell regeneration. Understanding how telomere maintenance is regulated in stem cells could have important implications for regenerative medicine and aging-related diseases.

Research has examined the use of telomere length as a biomarker of aging and age-related diseases. Shorter telomeres have been associated with an increased risk of developing various age-related diseases such as cardiovascular disease, diabetes and dementia. [6]

Advances in telomere research are also facilitating the development of personalized medicine approaches, as telomere length and telomerase activity can be used as markers to predict disease risk and determine optimal treatment strategies for individuals.

Conclusion

Thus, to summarize the above, it becomes obvious that the biological function of telomeres goes beyond protecting the ends of chromosomes from degradation and is of significant importance in the process of cell aging.

Further study of telomerase activity and telomere length in various diseases will allow us to develop modern approaches to their treatment and monitoring. Based on the state of the telomeres, the doctor can assess the risk of developing the disease, the prognosis of the course and outcome of the disease, and assess the effectiveness of the treatment. In addition, the study of genetic disorders of telomerase and telomeres that arise under the influence of exogenous factors will make it possible to influence risk factors for diseases and develop measures to prevent their development, as well as identify risk groups. Research into the mechanisms of functioning of telomeres and telomerase is a promising area for the development of new drugs. Thus, the telomere theory has become

the subject of many modern studies, the practical implementation of ideas about slowing down the aging process and a number of diseases. However, further research is required to resolve many unclear issues.

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