10.2478/mbs-2013-0027

Medical and Biological Sciences, 2013, 27/4, 5-10

REVIEW / PRACA POGLĄDOWA

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MECHANISMS OF CELL AGING IN CELL CULTURE

MECHANIZMY STARZENIA SIĘ KOMÓREK W HODOWLI KOMÓRKOWEJ

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S u m m a r y

A key element in the life of cells in culture is the number of cell divisions, not their life time in culture. Serially *in vivo* transplanted cells also exhibit a finite lifetime, which means that the cell aging is not unique only to a cell culture. There are theories suggesting that the aging of cells in culture may be associated with the aging of the organism from which they were obtained. Cells may stop dividing because of replicative aging, which is the result of telomere shortening. The aging process in cells may be induced by an intracellular process associated with shortening and uncapping of telomeres and environmental factors of a stochastic nature, among the most important of which is oxidative stress. The loss of telomeres beyond a critical value eventually induces antiproliferative signals that result in an aging. Telomeres give information about the end of replication; their function can be however recreated. Insertion of protein genes, comprised in telomerase, to aging human cells increases the length of their telomeres to lengths typical of young cells. The cells then exhibit all the characteristics of young nucleated cells. Telomerase is not only the central mechanism for regulating cells life, but it is also a mechanism that can be resumed, extending the replicative period of cells , comprising markers of gene expression characteristic of young cells, life. It is not known in what way replicative aging of cells is played by oxidative DNA damage, exposure to UV, oncogenes, which are independent of telomere shortening.

S t r e s z c z e n i e

Kluczowym elementem w długości życia komórek w hodowli jest liczba podziałów komórkowych, a nie czas życia w kulturze. Komórki przeszczepiane seryjnie in vivo wykazują także skończoną żywotność, co oznacza, że starzenie komórek nie jest charakterystyczne tylko dla hodowli komórkowych. Istnieją teorie, które sugerują, że starzenie się komórek w hodowli może być związane ze starzeniem się organizmu, z którego zostały one otrzymane. Komórki mogą przestać się dzielić z powodu starzenia replikacyjnego, które jest wynikiem skracania telomerów. Starzenie się w tym procesie komórek może być indukowane przez wewnątrzkomórkowy program związany ze skracaniem się i zmianami budowy telomerów (tzw. uncapping) oraz czynniki środowiskowe o charakterze stochastycznym, spośród których najważniejszym jest stres oksydacyjny. Utrata telomerów, po przekroczeniu wartości krytycznej, indukuje ostatecznie sygnały antyproliferacyjne, których skutkiem jest starzenie się. Telomery są więc zegarem replikacyjnego wyczerpania się, ich funkcja może być jednak odtwarzana. Wstawienie genów białek wchodzących w skład telomerazy do starzejących się komórek ludzkich powoduje wydłużenie ich telomerów do

długości typowej dla młodych komórek. Komórki wykazują wtedy wszystkie cechy charakterystyczne dla młodych komórek jądrzastych. Telomeraza jest nie tylko centralnym mechanizmem regulującym czas życia komórek, ale także mechanizmem, który może być ponownie włączony, przedłużając replikacyjny okres życia komórek, posiadających markery ekspresji genów charakterystycznych dla młodej komórki. Nie wiadomo, jaki wpływ na replikacyjne starzenie się komórek ma oksydatywne uszkodzenie DNA, ekspozycja na UV, onkogeny ras, które są niezależne od skracania się telomerów.

Key words: cell culture, aging, markers, telomeres
Słowa kluczowe: komórki w hodowli, starzenie się, markery, telomery

INTRODUCTION

A cell culture is characterized by a limited number of cell divisions. In the process of cells aging changes in cell morphology and inhibition of proliferation take place [1]. One of the most important issues in the research on aging is whether the changes observed in replicative aging can be correlated with the pathways and mechanisms of cell aging in situ. Another important and controversial aspect of replicative aging in cell culture is the process leading to the aging of phenotype achieved independently of proliferation [2]. It is suggested that phenotype aging is the final common pathway for actively dividing cells, in which signaling and/or metabolic imbalances may occur. It can be deducted that the cells may not be able to differentiate in vivo in a culture due to inadequate culture environment [2]. However, establishing a relationship is not required to use cell culture as a tool to carry out a research on aging mechanisms. There is evidence of the correlation of the aging cells in a culture with the aging cells in vivo, particularly in the processes present in both. To identify of cellular senescence in cell culture the authors used e.g. methods: telomeric repeat amplification protocol, senescence-associated β-galactosidase activity, morphological signs of aging.

HYPOTHESES

There are two main theories of cellular aging. One of them is defined as a damage model. It is characterized by impaired ability to repair deoxyribonucleic acid (DNA), causing randomly accumulated damages or mutations in DNA, ribonucleic acid (RNA) and the accumulation of altered proteins leading to the loss of proliferative capacity [3, 4] .

The second hypothesis assumes that aging is a genetically programmed process. Experimental evidence supporting theories of genetically programmed cell aging were provided in the researches of Pereira-Smith and Smith, and Sugawara et al [5, 6]. By fusing different immortal human cell lines Pereira-Smith and Smith suggest that the loss or inactivation of one of the many genes allows cells to avoid aging process [5]. If this hypothesis is confirmed it would allow the mapping of genes involved in cell aging [6, 7]. Preliminary mapping by Sugawara et al. of the gene of aging on the chromosome 1. was presented with the use of three independent experimental methods using human cells, among others, and immortal hamster cells [6]. Chromosome transfer experiments have shown that the introduction of a single copy of human chromosome 1, has renewed the program of aging in some immortal cell lines. The use of the technology of chromosome transfer has enabled mapping of the gene of aging on more than ten human chromosomes [7]. This method demonstrates that cell aging is controlled by genes that are activated, or whose functions are revealed at the end of the cell life. Faults in these genes allow the cells to avoid programmed process of aging and they become immortal. Perpetuation leads to progression of tumor cells. According to this hypothesis, aging is an active process controlled by definable genes; immortality is caused by damage or defects in these genes. This theory is based on the research conducted by Pereira-Smith and Smith, which showed that various immortal cell lines can complement each other when combined. This theory states that the introduction of a given human chromosome causes aging in some cell lines [5].

MECHANISMS AND MOLECULAR BASIS OF CELLULAR AGING

In vitro aging process is accompanied by loss of proliferative capacity, which results in a decrease in replication. It is necessary to identify biological markers of the aging process, which facilitate the identification of senescent cells in culture and in vivo. Some of these indicators are presented in Tab. 1. and include the insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), c-fos, beta-galactosidase. IGF-1 is produced by many cell types and plays an important role in the regulation of cell proliferation. Ferber and colleagues studied the production of IGF-1 which made it possible to detect the location of the IGF-1 receptor, which was explored in normal diploid fibroblasts [8]. The observations showed that the production of IGF-1 in messenger RNA (mRNA) decreased to undetectable level in the aging cells. Another biological marker, which is variously placed in aging cells, is the EGF. EGF signaling indicates the existence of disorders in non-proliferating aging human diploid fibroblasts below receptor binding. The lysates of young and aging WI-38 cells of the proteolytic activity targeted to the EGF receptor were compared [9]. The results of the experiments indicate that proteases cleave the EGF receptor, and the product is present only in aging fibroblasts. The production of proto-oncogene c-fos is important in the regulation of growth because it is a part of the transcriptional activator AP-1. In 1990, Seshadri and Campisi showed the loss of c-fos in aging WI-38 cells, which suggests that selective repression of c-fos led to the lack of proliferation [10, 11].

Table. I. *Markers of aging in cell lines [10, 11]*
Tabela. I. *Markery starzenia się w liniach komórkowych. [10, 11]*

Cell line	bio-markers	Description
WI-38, HS74, IMR-90	IGF-1	Senescent cells do not produce mRNA for IGF-1
WI-38	EFG	Altered form of EGF produced in senescent cells
WI-38	c-fos	Repressed in late passage cells
WI-38, IDH4, NHEK, CMV-MJ, HCA2	SA-β-Gal	Expressed in senescent cells but not in quiescent or terminally differentiated cells

It has been observed that the placement of different chromosomes can independently induce aging in the same line of immortal cells using the immortal lines derived from cancer cells mapping of senescence genes on chromosome 18 is enabled [12]. This finding suggests that there are many aging pathways and that immortal cells arise due to defects present in each of these pathways. The aging process can be activated by a single pathway, and the perpetuation of cells can be triggered by mutations within one of genes encoding proteins that are involved in this pathway. An alternative hypothesis is that the aging program is activated by several independent pathways. Immortal cells require at least one mutation in each pathway. Mutations that affect only one pathway do not immortalized cells but extend their lifetime. For example, infection with simian virus 40 (SV40) (which deactivates RB and p53 proteins) extends the life of cells but does not lead to the immortality of the infected cells [13]. Essential factors that lead to immortality of SV40-infected human cells are an additional genetic change or the loss of a chromosome 6 [14]. Antisense regulation of RB and p53 mRNA also results in longer cells life without immortalization [15]. Numerous hypotheses of aging are consistent with multistage immortalization observed in chemically induced pathway [16]. Furthermore, the inability to assign immortal cells to the same group confirms this hypothesis [17].

MARKERS AND SENESCENCE GENE

Using the technique of chromosome transfer suggested by Sugawara et al human genes of aging were mapped. Chromosome 9p21 which belongs to the region where p16 gene of aging, which relates to this region, was mapped enabling the activation of the aging process in the region of chromosome [18]. The protein p16 is an inhibitor of cyclin-dependent kinases CDK4 and CDK6; it is a major regulator of the G1 phase cell cycle. In addition, p16 is mutated in many immortal cancer cells. It has been shown that p16 is modulated during cellular aging and its activity is enhanced in aging human cells. It acts as the main inhibitor of cyclin-dependent kinases in aging cells [19]. As p16 is mutated in many cell lines, and reintroduced to immortal cell lines pl6 causes induction of the cellular aging process, it can be concluded that pl6 is the gene of aging mapped to 9p21 [20].

Two other genes of the control of cell cycles p53 and Rb also demonstrate properties related to aging [14]. The p53 protein is an important factor regulating the passage of the cell through G1 phase. In response to DNA damage accumulation of normal (wild) p53 protein takes place, resulting among other things in, transcriptional induction of p21 protein, which is an inhibitor of cyclin kinases [21]. Accumulation of p53 protein is not due to de novo gene transcription. In response to DNA damage the activation of a cascade of kinases and other proteins, including ATM, ATR, Chk1, Chk2, CDC25C, and the interaction of p53 with mouse double minute 2 homolog (MDM2) occur [22, 23]. The result of this is the reduction of the degradation of p53 protein and thereby the increase in the level of this protein in the cell. Inhibition of G1 phase kinases activity results in the accumulation of unphosphorylated Rb protein, which in turn causes the blockage of the cell cycle. Reintroduction of genes into some immortal cells inhibits the growth of them and leads to morphological changes characteristic of aging cells [24, 25]. In normal cells p53 and Rb proteins perform a negative role of regulators in the cell cycle, and that is why these proteins are controlled by other proteins, which enables the proper continuation of the cell cycle. In the aging cells the program is activated upon inhibition of DNA synthesis in the cell cycle, the consequence of which is the growth arrest in G1 phase. Rb and p53 are involved in one or several pathways that activate or influence the aging process. Deletions or mutations occurring in p53 and Rb genes may cause inability to activate the process of aging. p53 and Rb in the proper form is present in some immortal cells, and the genes that control their phosphorylation or other posttranslational modifications may be defective [24].

REPRESSION OF THE ENZYME TELOMERASE

Not only cell cycle regulation is associated with aging process. Different functions of the aging gene that are linked with repression of the enzyme telomerase, which is responsible for the maintenance of telomeres in most immortal cell lines, is also involved. Telomeres are specialized structures at the ends of chromosomes, which consist of tandem repeated DNA sequences - (TTAGGG) and proteins associated with them such as telomeric repeat binding factor (TRF1) and TRF2 [26]. Telomeres function as a mitotic clock by setting the cell life [27]. In normal human somatic cells, which do not show telomerase activity, the telomeric DNA progressively stops shortening in the process of cell division. This results from the inability of DNA polymerases to replicate the linear ends of DNA molecules, or it dependents on some other mechanism of the final DNA degradation [27]. It is assumed that the cellular aging process begins when the telomeres reach a critically short length [27]. Immortal cancer cells have mechanisms that compensate for telomere shortening, usually by activating of telomerase, allowing them to stably maintain their telomeres and grow indefinitely [28]. Three components of the human enzymes of telomerase were identified: human telomerase RNA the component of human telomerase RNA component (hTERC), also known as hTR, which acts as a template for telomere repeat synthesis, telomerase boundprotein-l/telomerase protein component 1 (TP1/TLPI), which is similar to the Tetrahymena telomerase protein p80. and the catalytic subunit of telomerase reverse transcriptase and containing human telomerase reverse transcriptase (hTERT), also known as hEST2/hTRT, which were isolated on the basis of their similarity counterparts in yeast and Euplotes aediculatus [29].

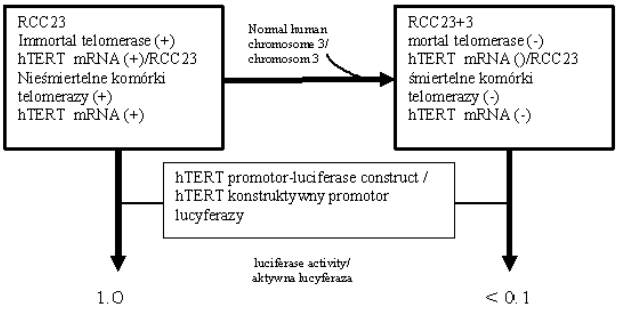

Fig. 1. *Transcriptional repression of hTERT gene by a telomerase repressor gene on chromosome 3 [29]*

Ryc. 1. *Represja transkrypcji genu telomerazy hTERT przez genu represorowego na chromosomie 3 [29]*

Of interest is the way in which components activate and regulate the human telomerase and how they are regulated by factors that may play a role in aging and cellular immortality and carcinogenesis. hTERC and hTEPI are present in normal cells: mortal and immortal. hTERT expression is detected in telomerase positive cells but not detected in telomerase negative cells. Furthermore, the introduction of the hTERT gene promoter constructively deprive them of telomerase activity thus is the catalytic subunit of telomerase. hTERT expression and repression leads to wrong regulation of telomerase, and thereby adversely affect the viability of normal cells [30].

CONCLUSIONS

Nowadays it is known that the cells cease to divide due to old age or due to terminal differentiation (e.g. nerve cells). It is assumed that the aging of cells also occurs in vivo and that the life expectancy and replicative lifetime of cells are under common genetic control. There are suggestions that the aging of cells prevents the formation of tumors in them. On the other hand, the old human fibroblasts stimulate the growth of epithelial tumor cells, but have no effect on normal cells. This effect may be caused either by a direct contact of old fibroblasts and tumor cells as well as by soluble factors produced by old fibroblasts. This phenomenon is referred to as senescence-associated secretory phenotype (SASP). The main hypothesis of cellular aging is the shortening of telomeres [27]. This theory constitutes the explanation of the mitotic clock in the replication process of cellular aging. But there is also evidence to suggest that signals other than telomere shortening may be the cause of cell aging. Cellular aging is included by oxidative stress, and terminal differentiation as well as changes in DNA methylation. It is possible that oxidative stress causes aging of cells by affecting the rate of telomere shortening. Further studies will help to determine whether telomere shortening is the main mechanism of induction of cellular aging, or if there are other telomere-independent inducers also involved in this process. The cellular process of aging is controlled by many genes. Important in the regulation of genes in this process are mutated and deactivation immortal cancer cells that have avoided aging. This process can be restored in these cells by the introduction of normal chromosomes, enabling the mapping and cloning of genes. The basic functions of these genes are helpful in explaining of regulation, cell cycle control and regulation of telomerase. All these factors are important inducers of cellular pathways of aging.

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Received: 13.11.2012
Accepted for publication: 14.10.2013