ANALYSIS OF GENETIC MECHANISMS OF AGING BASED ON THE LATEST RESEARCH

Dariusz Polański[*1], Anna Bogucka-Kocka[2], Robert Kaczmarczyk,[3] Anna Makuch[4], Janusz Kocki[1]*

[1] Department of Clinical Genetics, Medical University of Lublin, Poland
[2]Chair and Department of Biology and Genetics, Medical University of Lublin, Poland
[3]Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Lublin, Poland
[4]Students Scientific Society of the Chair and Department of Biology and Genetics, Medical University of Lublin, Poland

[*]Correspondence author e-mail: darpol2@onet.eu

S u m m a r y. Aging is a complex and unavoidable process that can be modified by the environment, involving many biochemical and molecular mechanisms in cells, leading to functional impairment and pathological disorders. Repair systems are not able to get rid of it, resulting in abnormal tissue function, increased susceptibility to disease and consequently lack of homeostasis of the organism and its death. Aging is also a constant decrease in the ability to regenerate lesions and overcome exogenous stress, leading to the diseases and degenerative changes. Genetic theories of aging point to the molecular mechanisms of inheritance and the regulation of gene expression and variability. The hypothesis of a genetic clock in cells is based on the limited number of cell divisions known as Hayflick limit. Damage to genome increases with age despite solid repair mechanisms, leading either to apoptosis or to ceased proliferation and acquiring aging phenotype. Epigenetic factors also affect the regulation of cell survival without directly influencing the DNA nucleotide sequence. Telomere shortening theory and programmed death theory (apoptosis) also attempt to explain the phenomenon of life time limit. Accelerated aging syndromes in progeria show that DNA damage is responsible for senescence. Damage to the DNA caused by external mutagens or endogenous factors may become a signal to begin the path of cellular aging.
K e y w o r d s: aging, senescence, genetic theories, telomeres, genome, molecular mechanisms

INTRODUCTION

Aging of living organisms is a phenomenon that everyone observes, and sooner or later it also becomes our personal experience. We share both negative and positive effects of aging, progressive loss of efficiency and age-related illnesses, along with increasing experience, wisdom and life achievements. Biological aging is visible in all living world and has always been treated as something inevitable and natural. Along with improved living conditions, new drugs and vaccination against infectious diseases, there has been a gradual prolongation of human life, both average and maximum duration [1]. Constant pace of life extension is also due to improved medical care of the population, especially over the age of 65, and effective prevention and treatment of age-related diseases [2]. For women in Japan, the expected average life expectancy is currently 85 years and it is the highest rate of average survival in developed countries. At the age of 65, one of these women out of 20 has a chance to celebrate a hundredth birthday [3]. In the United States the group of centenarians is the fastest-growing population of people, in 1940 there were 3,700 such people, and in 2006 about 61,000 [4].

DEFINITION OF AGING

Aging is a complex and unavoidable process, modified by the environment [5], involving many biochemical systems and reactions including molecular transformations in cells, manifested by

various cell, tissue and organ changes occurring throughout the body and leading to functional impairment and pathological disorders [6]. Accumulation of intra- and extracellular damages in the organism becomes visible, repair systems are not able to quickly reduce and get rid of them and to maintain the constant level of homeostasis, resulting in abnormal functioning of tissues, increased susceptibility to diseases and consequently disturbance of the internal balance of organism leading to its death [7].

It has been also found that the mortality of some species, like giant tortoises for instance, does not increase with age. The reproductive abilities of these animals surprisingly do not change over the years, thereby neglecting the universal rules of the aging process. This phenomenon, unique in the world of multicellular organisms has been termed as the negligible senescence [8].

Aging can still be described as a permanent decrease in the ability to regenerate lesions and overcome exogenous stress related to the environment in which the organism lives, thereby increasing the incidence of diseases and degenerative changes [9]. In 1997 scientists established a new term of senescence called successful aging, including patients with a low risk of morbidity and of impaired ef□ciency, with a relatively high level of mental and physical health and with active and creative attitude towards life [10]. A quantitative evaluation of the successful aging has been developed by determining the degree of physical and functional disorders at a given age named "frailty". This term covers the whole spectrum of psychophysical and behavioral changes in the elderly organism [11]. Further investigators de□ned it as an age-related clinical syndrome determined mainly by weight loss, general fatigue, muscle weakness, decreased walking speed, and poor physical activity [12]. The frailty index (FI) has been later introduced broadening the assessment of aging by the percentage of accumulated health de□cits in a number of different vital functions and deviations in the patient's laboratory tests [13].

GENETIC THEORIES OF AGING

Genetic theories seem to be very important as far as aging is concerned, emphasizing molecular mechanisms, focused on the processes of inheritance and the principles of regulation of gene expression and variation in the organism, influenced by various factors of the external environment. The genetic material of a cell is not protected permanently. Various disorders and malformations can occur, induced by physical or chemical mutagens or take place spontaneously [14]. These are usually point mutations that convert a single nucleotide to another. It can affect somatic cell systems and may be sometimes lethal. Genetic malformation may also affect reproductive cells, then it is inherited affecting the evolution of the whole genome.

Various factors, both endogenous and exogenous, affect the organism and can lead to DNA damage. The endogenous ones include the action of reactive metabolites arising in cells, abnormalities in recombinant DNA and spontaneous replication errors associated with incorrect nucleotide pairing. Replication errors, in addition to point mutations, can also lead to other mutation types like deletions or insertions shifting the translation phase. It can also generate DNA sections with fewer or more repeat units. Such changes may phenotypically respond to certain neurodegenerative disorders, such as Huntington's disease [15].

Exogenous causes of mutation include chemical factors such as various aromatics and alkylating agents, base analogues, deamidating and intercalating agents, and many more. Mutagenic physical factors include ionizing and non-ionizing radiation, mainly ultraviolet (UV), and high temperature. Exogenic factors can directly or indirectly induce various changes in the genetic material, like formation of transverse links within a DNA strand and between strands, single stranded or double stranded DNA breaks, modi□cations and loss of nitrogen bases, oxidation and alkylation, formation of cyclobutyl dimers and other photodamages [16].

Every day genome is exposed to thousands of exogenous lesions and errors occurring during endogenous replication of genetic material. The cell has, however, ef□cient repair systems without which it would not be able to maintain its functions. Direct repair eliminates DNA tears by DNA ligase reaction, alkylated nitrogen bases are reactivated by alkyl transferase reactions transferring the alkyl group from the nucleotide to their molecule. On the other hand, so called cleavage is a system that allows the removal of an incorrect base (base excision repair, BER) or the whole nucleotide (nucleotide excision repair, NER). Repairs of erroneously paired nucleotides are based on the effect of an MMR (mismatch repair) enzyme complex, MutH, MutS and MutL proteins, which recognize and cleave the improperly paired base [15].

Double strand breaks (DSBs) are particularly difficult to remove and dangerous to cells, they are often caused by various chemical mutagens and by ionizing radiation. DSBs are repaired by linking the ends of DNA, that are not homologous to each other (nonhomologous end joining, NHEJ), with the surrounding of a specific enzyme complex, DNA ligase IV, kinases, Ku-protein molecules and XRCC4 factor. Restoration of the correct genetic information is also possible due to the enzyme complex of the recombinant repair system and the exchange of correct sequence information between two sister DNA strands from homologous chromosome pairs [17].

Despite the existence of repair mechanisms and prevention of malformations, the amount of various lesions in the genome grows with age. Continuous exposure to harmful external environmental factors, genomic damage caused by viruses or reactive oxygen species, endogenous replication errors, and spontaneous modifications of nitrogen bases result in intracellular collection of point mutations, chromosome deletions and translocations, telomere shortening and polyploidization. Eventually, the number of these anomalies outweighs the ability of repair processes leading either to cell apoptosis in case of very large lesions or arrest proliferation, and finally a specific phenotype of the aging cell develops [16].

Structural balance in the genome and regulation of life expectancy are also influenced by epigenetic factors that affect genetic information at the level of mitosis, and do not directly affect the nucleotide sequence in DNA. The methylation of cytosine in DNA occurs mainly in certain areas of DNA, and its level significantly decreases with age. In addition to the general occurrence, demethylation of specific genes such as the MYC gene and hypermethylation of other gene promotors, such as transcriptional factors, can also be observed [18].

miRNAs are important elements of epigenetic control over cell genome. They are composed of 21-23 nucleotides. These short, non-coding regulatory RNA sequences bind to their own specific mRNAs and block their translation, thereby preventing the expression of corresponding genes and the production of protein products. In some age-related diseases miRNA overexpression has been observed. It has also been shown that miRNAs inhibit the expression of many genes involved in the regulation of many cellular processes and they are important in controlling life span and processes of aging [20].

Abnormalities in epigenetic post-translational modifications of histone proteins, mainly acetylation and histone methylation, have been observed in older cells. As a result senescence associated heterochromatin foci (SAHF) form. They are mainly located near the promotor sequences of genes encoding S-cell cycle regulators of the cell cycle and are built of proteins such as HMGA (High Mobility Group A), HP-1 (heterochromatin protein-1), histone macrophyll H2A, and histone H3 protein [19].

This age-related heterochromatin is considered to be a specific marker of so called replicative cellular aging, which is a direct consequence of the depletion of somatic cell division potential [22].

The theory of a limited number of cell divisions, formulated in the 1960s, clearly pointed to the causes of cellular aging in the living organisms. Leonard Hayflick and Paul Moorhead have proven that *in vitro* cells are not immortal, they may divide a certain number of times (Hayflick limit) and continue to grow old in breeding cultures [21]. According to recent knowledge, aging is much more complicated, there are alternative paths of cell senescence, and the limitation of cell division is one of the mechanisms. Cellular aging as a cause of aging of the whole body and a source of diseases in the elderly organism becomes a hypothesis increasingly accepted by many researchers [22].

At the end of the twentieth century the hypothesis of a limited number of cell divisions was supplemented by the theory of telomere shortening, which declares that the reason of aging is due to the gradual decrease in the length of DNA sequences at the ends of eukaryotic chromosomes. The structure of telomere DNA consists of minisatellite sequences with the repeated copies of the 5'-TTAGGG-3'. At each end of the chromosome, there are several hundred copies of this segment, primarily for preserving the integrity of genome by preventing chromosome endings from linking together [15].

Telomeres are supported by a group of proteins called shelterins, which include TRF1 (telomeric repeat binding factor 1), TRF2 (telomeric repeat binding factor 1), TIN2 (TRF1- interacting nuclear protein 2), Rap1 (repressor-activator protein 1) and POT1 (protection of telomeres 1). They help maintain specific structure of telomeres, ensuring genomic stability. They also participate in the control of telomerase enzyme activity [23].

Shelterins are connected with each other by protein-protein bonds and protein-DNA bonds. The specific construction of telomers with the T-loop and TRF2 protein prevents unwanted biochemical

pathways. TRF1 protein controls telomerase activity at individual chromosome ends by affecting the chromatin structure. Failure of the TRF1 leads to the formation of an open chromatin structure, which allows telomerase enzyme to actively prolong telomeres. On the other hand TRF1 protein in a collapsed form enables to remove telomerase and stops telomeres from extending. Activation of the telomerase enzyme is also dependent on other components of the *shelterin* complex, like POT1, TPP1 and TIN2 [24].

The synthesis of telomere DNA in cell takes place primarily in the regular DNA replication process. However, during the amplification of DNA strands telomeres are shortened because of the mechanism called unequal replication of both strands of DNA, catalyzed by an enzyme complex comprising helicase, primase, DNA polymerase and exonuclease enzymes [26].

The leading strand of DNA is formed continuously from the 3 'to the 5' end. Delayed strand however is synthesized from the 5 'to 3' end and fragmented by RNA primers initiating the process. New sections of the polynucleotide chain known as Okazaki fragments are then joined together by ligase and the nuclease enzyme removes unnecessary primers [25]. The newly synthesized fragment of the delayed strand is shorter than the original. This is so called end replication problem due to the fact that DNA polymerase is unable to replicate the end of the delayed strand. Then, a shortening of the DNA sequence and telomere length loss occurs [27].

To complement the deficiencies of the nucleotide sequences generated during replication, DNA synthesis takes place in a completely independent telomerase-catalyzed process. This enzyme, i.e. a reverse transcriptase, is responsible for maintaining constant length of telomeres in the cells. The telomerase complex is composed of telomerase reverse transcriptase (TERT) subunit, telomerase RNA component (TERC) subunit, which is the RNA template for telomerase, and the associated proteins [28].

Regulation of telomerase activity is a complex mechanism, it takes place on many levels and is modulated by active proteins. Malformations in the construction of telomerase subunits cause the appearance of hematological proliferative diseases syndromes or neurodegenerative pathologies [29].

Telomerase activity is closely related to the type of cells tested and to the stage of their biological development. It does not appear in all kinds of cells in the organism. The enzyme is very active in embryonic and pluripotential cells. The activity of telomerase is equally stable and high in reproductive cells. Increased level of telomerase has been observed in tumor cells where it prevents cell conversion to the replication aging phase and enables multiple divisions. In these cells the length of the telomeres is usually much larger than normally, due to the hyperactivity of telomerase. As far as somatic cells are concerned usually no telomerase activity is observed, but when there is a certain enzyme level at the beginning, it gradually decreases with age [30].

In spite of the telomerase activity and additional mechanisms needed to extend the ends of chromosomes after unequal replication it is not possible to maintain the original telomeres length in somatic cells for long. Over time, these sequences are shortened and cells tend to replicate resting state or apoptosis. Achieving a critical account of about 100 and less telomeric repeats aging processes start and it seems to be directly related to the Hayflick limit (about 80 cell divisions, depending on the cell type and its biological status) [31].

Shortening of telomeres can also be accelerated. For instance under the influence of oxidative stress, reactive oxygen species acting directly on the genetic material, damage to nucleic acids, single or double DNA strands cracks, and nitrogen base modifications occur. Damage to phosphodiester bonds, the formation of crosslinking bonds between nucleic acids and proteins and modifications of internal carbohydrates are also possible. All of these changes cause degeneration and structural damage to the genetic material and along with the laminopathy lead to the damage of nuclear and mitochondrial DNA, inducing accelerated telomere shortening and premature aging [33].

The progerias, which are accelerated aging syndromes, provide convincing and direct evidence to the theory, that DNA damage caused by even single mutation in the genetic material can be the cause of cellular aging. and in macroscopic scale is responsible for the phenotype of senescence, along with chronologically occurring illnesses in the elderly life. Premature aging syndromes are very rare pathologies, their common feature is the occurrence of unnaturally early age-related changes. In that case cells have a high level of damages to nucleic acids, they have shorter telomeres, and therefore have a lower replication potential [38].

These diseases are known as the Werner's syndrome, Cockayne syndrome, Hutchinson's – Gilford' progeria, Fanconi's anemia or Bloom's

syndrome. Patients suffering from Hutchinson-Gilford Progeria Syndrome (HGPS) live approximately 12-13 years, they carry the mutation of the LMNA gene in chromosome 1 that decides to produce a defective and shortened form of the lamina A. The normal lamina A is a protein that guarantees the correct shape of the nuclear membrane, participates in numerous processes like regulation of transcription, it decides about the proper structure of nuclear chromatin, repair and replication mechanisms of DNA [35].

Patients with Werner syndrome (WS) live longer (average 47-55 years), compared to those with HGPS, the first symptoms of this disease appear at the end of adolescence. WS is the result of a mutation in the gene encoding WRN protein belonging to the Helikaz RecQ group, responsible for proper DNA stranding, successful replication, effective transcription and repair of nucleic acids to maintain telomeric sequences in a good condition [36].

Aging cells, despite of the lack of division, are still metabolically active, they start to secrete some compounds earlier not produced, such as pro-inflammatory cytokines and growth factors. Thus they acquire a number of features that make their general image very characteristic, called senescence associated secretory phenotype (SASP) [40]. Aging cells also accumulate products of metabolism, generate errors in gene expression and wrong repairs, increase free radicals and number of errors in protein synthesis and replication. The increased amount of mutations in aging somatic cells brings the potential risk of neoplastic transformation [32].

Senescence Associated Heterochromatin Foci (SAHF) are specific markers of aging, they are heterochromatin groups located in aged cells. They are markers for the second type of senescence, called stress-induced premature senescence (SIPS). It occurs in living cells both *in vivo* and *in vitro* and is induced by various chemical, biological and physical factors causing damage to nucleic acids. Accelerated aging (SIPS) does not depend on telomeres shortening or on the cell's inability to divide. Reactive oxygen species can stimulate SIPS, aggressively destroying the structures of the genome often resulting in single-stranded DNA cracks that tend to transform into double-stranded nucleic acid tears at the time of replication. Oxidative stress induces double stranded DNA cracks also in telomere sequences that are particularly sensitive to it probably due to high guanine content. These damages are also the reasons for the shortening of telomeres [42].

Accelerated aging described as oncogene-induced senescence (OIS) occurs in living cells by expression of many oncogenes, such as MOS, Ras, BRAF or MEK. Expression of oncogene and production of Ras protein leads human fibroblasts to aging *in vitro*, as demonstrated by Manuel Serrano in his pioneering work in 1997 [37]. In addition to normal cells, SIPS also occurs in pathologically altered cells like cancer cells undergoing chemotherapy cycles [39].

CONCLUSIONS

Numerous studies have been conducted and many theories have been developed to explain the basics of senescence process in living organisms. Genetic theories emphasize the molecular mechanisms centered around the processes of inheritance as well as the principles of regulation of genes expression in the organism influenced additionally by environmental factors. However, variety of life expectancy in different species speaks for its genetic regulation so the vision of longevity and aging without age-related diseases encourages further exploration.

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