BIOGERONTOLOGY

Role of Peptides in Epigenetic Regulation of Gene Activities in Ontogeny

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The authors develop a new concept most fully reflecting the evolutional and biological role of peptides in the organism. Wide spectrum of peptide effects realized through regulation of the expression of certain genes is aimed at the maintenance of homeostasis, inhibition of the genetic aging program realization, and lifespan prolongation.

Key Words: *peptides; epigenetics; ontogeny; aging; age-associated disease*

Studies of molecular genetic mechanisms of individual development and aging are an important trend of modern medicine. By the present time it is known that some genes are essential for the lifespan and longevity [12]. Many-year studies of the molecular and genetic mechanisms of aging have revealed in what way the genes modulate the lifespan and aging of certain organisms. There are numerous examples of how differences in the gene expression and activities lead to differences in the lifespans of related organisms because of age-associated changes at the genome level. However, the genetic mechanisms of these disorders remain unknown for the majority of species. It seems that the genetic mechanisms of aging are much more intricate and their species variations are much greater than the genetic mechanisms of development. However, no principally new biological mechanisms, other than those working in the course of the organism development, have been suggested to explain the aging phenomenon. We know many examples when biochemical and cell functions remain unchanged and not disturbed throughout the entire lifespan. Studies in developmental biology have shown that the majority of human somatic cells possess a certain proliferative potential throughout the lifespan and retain the differentiation potential. When these functions reduce with aging, it is possible to modulate the processes by modulating gene expression [10]. Studies of the genetic mechanisms of aging and of age-specific disease development suggest the regulatory therapy philosophy: the use of transcription modulators inhibiting and restoring genetic changes emerging with age. This implies the knowledge of the genome topography and the developing shifts and use of substances with selective effects on various regulatory mechanisms of genes [7,10,11].

The threshold lifespan of animal and human species is about 30-40% longer than the mean lifespan. This can be explained by exposure to various unfavorable factors leading to changes in gene expression and structure paralleled by disorders in protein synthesis and reduction of body functions (Fig. 1).

The processes explaining these changes have been intensely studied in recent years. The epigenetic mechanisms determining the changes in gene expression and activities without modifying DNA structure [3] are now in the focus of attention.

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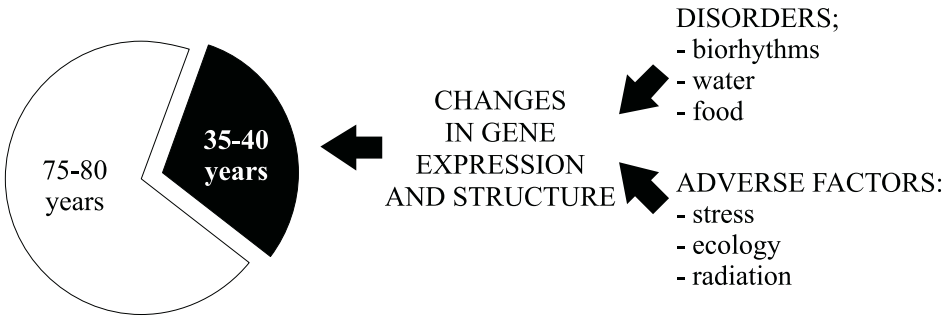

Fig. 1. Species-specific lifespan of a human being and the biological reserve. Light section: mean lifespan of a modern human (early aging); dark sector: biological reserve of human life.

It is now accepted that aging starts long before birth. The factors to which an organism is exposed throughout all stages of prenatal ontogeny can initiate the changes aimed at improvement of adaptation to postnatal environmental conditions or lead to development of various pathological shifts in the postembryonic development, age-associated diseases, and early aging [4].

The genome imprinting makes an important contribution to the development of these changes. The genome imprinting is based on specific epigenetically regulated structural and molecular changes in certain sites of chromosomes, occurring during the formation of male and female sex cells and leading to stable functional differences in the expression of homologous genes in the progeny. Methylation of DNA cytosine bases switching off the gene transcription and specific for individuals of different genders is assumed to play the key role in this process. This mode of gene regulation indicates a non-equivalent contribution of the parents to the genome of the progeny. Importantly that imprinted genes are essential not only for regulation of the pre- and postnatal growth of the organism, but are also involved in the regulation of behavioral reactions and in the development of such multifactor diseases as cancer, diabetes mellitus, atherosclerosis, asthma, schizophrenia, *etc.* [6].

Thus, aging is a process of genetic and epigenetic interactions at all biological levels, in which the epigenetic regulation plays an important role in determination of the emerging phenotypical differences. The mechanisms of epigenetic regulation also play the key role in the development of aging-associated diseases and explain the relationship between human genotype and the environment. The epigenetic changes are caused primarily by DNA methylation [2]. B. F. Vanyushin *et al.* [14] have revealed the tissue, subcellular, and age specificity of DNA methylation. It has been found that DNA methylation pattern of cancer cells is modified [13].

It was found that aging was paralleled by disorganization of the peptidergic system of body function regulation. Studies of organ and tissue age-specific involution detected reduced production of physiologically active peptide substances and reduction of protein synthesis intensity, which attests to an important role of peptides in the regulation of mechanisms of aging. The peptide system is regarded as a universal in neuroimmunoendocrine interactions [5]. In parallel, peptide regulation of physiological functions involves tissue-specific peptides maintaining the tissue homeostasis. Peptide bioregulators are present in various cells and tissues, are formed during limited proteolysis, are characterized by a wide spectrum of biological effects, and coordinate the development and functions of multicellular systems. Despite the multilevel hierarchy, the mechanisms of peptide regulation of homeostasis perform a common function: coordination of the biosynthetic processes in cells by modulating gene expression.

Peptide regulation of homeostasis is an important component of the long chain of processes leading to aging of cells, tissues, organs, and the body in general. Morphofunctional equivalents of aging are involution of organs and tissues, primarily those belonging to the diffuse neuroimmunoendocrine system. Age-specific hypoplasia and in many cases degeneration of the pineal gland, thymus, cortical and subcortical neurons, retina, vascular wall, sexual organs were reported [1]. Involutive changes in organs and tissues during aging lead to reduction of their function. At the cellular level, this manifests primarily by disorders in specific protein synthesis. The key fact is reduction of regulatory peptide synthesis in cells during aging and changed sensitivity of target cells to them. Wide spectrum of biological effects of these substances suggests that reduction of their production leads to disorders in the peptide regulation mechanisms and gradual cessation of the functions in an aging organism. On the other hand, the production of some regulatory peptides increases with age. These changes in an aging organism can result from a total imbalance of the regulatory factors or be a manifestation of some processes compensating for the age-specific shifts. A unique

feature of peptide regulation of homeostasis is polypeptide processing: by peptidase activation it initiates the production of the needed amount of short peptide fragments with biological activities higher than those of the initial compounds in the needed place at the needed moment. The activities of cellular endo- and exopeptidases in animals reduce with aging. This is paralleled by the formation and accumulation of significant amounts of peptides resistant to the available peptidases in the cells. However, the causes of peptide accumulation in old cells remain not quite clear. Hence, analysis of the spectrum of age-specific disorders in the peptide regulation of homeostasis clearly shows the common trend: the synthesis and secretion of regulatory peptides and the sensitivity of the target cells to them decrease during aging.

Modern development of gerontology provides new data extending our knowledge of the mechanisms of aging and suggests new possibilities for creation of geroprotective means physiologically adequate for humans. Comprehensive studies of the peptide effects indicate that compounds of this class most fully meet these requirements [7].

The classical data on the normalizing effects of peptides on age-associated hormonal-metabolic and immunological changes are now supplemented by the results of studies of peptide effects at the genome level [10]. Studies of the peptide biology have shown that these substances are directly involved in tissue-specific regulation of gene expression and biosynthesis. Peptide regulation inhibits the accumulation of pathological changes (DNA aberrations, mutations, malignant transformation, *etc.*) in the cells and stimulates the reparative processes aimed at cell homeostasis recovery.

Numerous studies [8,10] have demonstrated that endogenous peptides are involved in the regulation of cell differentiation and proliferation, modifying the genome functional activity and protein synthesis processes, depending on the status of the multicellular system. If we regard the manifestations of life as an evolutional process of genetic information exchange and reproduction, the significance and origin of peptide bioregulators become clear. It is known that cell mediators play the key role in regulation of gene expression at the early stages of multicellular system development. Therefore, autoregulation mediator mechanisms are among the first to appear in the cells which have no species specificity but have cellular specificity due to which they regulate the multicellular organism developmental mechanisms. The mediator systems formed at the early stages of evolution seem to retain their significance after cell specialization, serving as important mechanisms regulating the gene activities. Disorders of peptide regulation reduce cell and tissue resistance to destabilizing factors of the environment and inner media. This can serve as one of the causes of organ and tissue involution, disease development, and rapid aging. Use of peptide bioregulators under conditions of disordered cell homeostasis leads to recovery of the functional activities of the respective physiological systems and organs. By the present time regulatory peptide complexes have been isolated from all cells, tissues, and biological liquids of the body. Physicochemical studies have shown that they differ by composition, molecular weights, and electrochemical characteristics of the components.

Experimental and clinical studies of the peptide effects on age-associated changes have demonstrated their high geroprotective activity [8]. It has been found that the expression of genes whose products are involved in inflammatory reactions increases with aging, while the expression of genes involved in energy supply to the cell, cell differentiation, and cell proliferation regulation is reducing. It is noteworthy that the inflammatory reactions make an important contribution to the pathogenesis of age-associated changes in human organism, among which atherosclerosis and Alzheimer's disease are of primary significance. Liability to inflammatory processes is a result of immune imbalance associated with the predominant reduction of T-dependent immune reactions. This reduces the resistance to many infections and cancer, the incidence of which increases with aging. One more factor promoting a higher incidence of tumor diseases is reduction of cell proliferation and differentiation regulation, this promoting tumor growth and impairing tissue homeostasis, regeneration, and normal functioning of organs and tissues. These functions reduce because of disorders in the mechanisms of energy production by the mitochondria. Disorders in the mitochondrial functions lead to more intense production of active oxygen species, factors promoting tissue injury and development of cancer, atherosclerosis, and age-associated neurological disorders. Of age-associated changes in differentiated tissues, of special importance are the processes in the neuroendocrine organs, because they modulate the functions of other systems regulated by respective hormones. With all this in view, we can evaluate the results of studies of molecular genetic mechanisms of peptide effects aimed at prevention of aging.

It is known that expression of many genes (including those determining the production of regulatory peptides) changes with aging. The resultant regulatory imbalance can be compensated or corrected by peptide bioregulators causing normalization of the functions of certain organs and tissues and exhibiting positive systemic effects, such as improvement of energy supply to tissues, reduction of active oxygen species formation, prevention or correction of age-specific disorders in the immune, endocrine, and other functions.

Studies of the peptide structure and functions are of crucial importance for the development of concepts on the mechanisms of the geroprotective effects of these substances. Experimental studies of short peptides have confirmed the hypothesis according to which the peptide regulators regulate the gene expression and protein synthesis in the cells. Studies of the effects of synthetic Glu-Trp, Lys-Glu, Ala-Glu-Asp-Gly, and Ala-Glu-Asp-Pro peptides have shown that injections thereof to mice modify the profiles of gene expression in the myocardium and brain. Changes in the gene expression seem to be the most probable cause of physiological effects of peptides, such as melatonin secretion rhythm recovery in response to injection of Ala-Glu-Asp-Gly peptide to old rhesus macaques or prolongation of the functional and morphological integrity of the retina of the eye after injection of Ala-Glu-Asp-Gly peptide to rats with hereditary pigmented degeneration of the retina [7]. The effects of Lys-Glu and Ala-Glu-Asp-Gly peptides on the expression of IL-2 and *c-fos* genes presumably largely determine the immunomodulating, oncomodifying, and stress-protective effects of these peptides. The results of studies of Lys-Glu and Ala-Glu-Asp-Gly peptide effects on carcinogenesis in transgenic mice confirm this hypothesis and indicate the effects of these peptides on the expression of HER-2/neu (breast cancer) gene. The relationship between free radical oxidation and aging processes suggests the significance of the antioxidant and antimutagenic effects of Lys-Glu and Ala-Glu-Asp-Gly peptides. The mechanisms of geroprotective effects of short peptides, consisting in chromatin activation in blood lymphocytes of senile patients, have been disclosed for the first time. This opens new vistas in prevention of rapid aging and correction of age-associated diseases. The effects of Ala-Glu-Asp-Gly peptide consisting in telomerase activation, elongation of telomeres in various cells, and prolongation of diploid human cell vital cycle by overcoming the Hayflick limit are an important result of modern biogerontology and a fact which confirms the good prospects of further creation of geroprotective peptide drugs [10].

It has been found that short peptides (di-, tri-, and tetrapeptides) interact in a complementary mode with the specific DNA binding sites on the gene promoter regions causing separation of DNA chains and stimulating RNA polymerase. Detection of the phenomenon of peptide stimulation of gene transcription suggests natural mechanism of physiological functions maintenance based on interaction of complementary DNA-regulatory peptide interactions. This process underlies the development and functioning of living matter [8] (Fig. 2).

The majority of the studied peptides bind to two- and single-strand deoxyribo-oligonucleotides containing CG and CNG sequences, targets for DNA methylation in eukaryotes. Peptides specifically modulate the effects of eukaryotic CG and CNG site-specific endonucleases (WEN1 and WEN2) on DNA *in vitro*, depending on the DNA methylation status. In the majority of cases, the peptides inhibit significantly DNA hydrolysis by these enzymes. Presumably, modulation of endonuclease effect on DNA hydrolysis is realized due to site-specific peptide-DNA binding, protecting the DNA from enzymatic hydrolysis. It is assumed that peptide binding to the DNA promoter CG or CNG

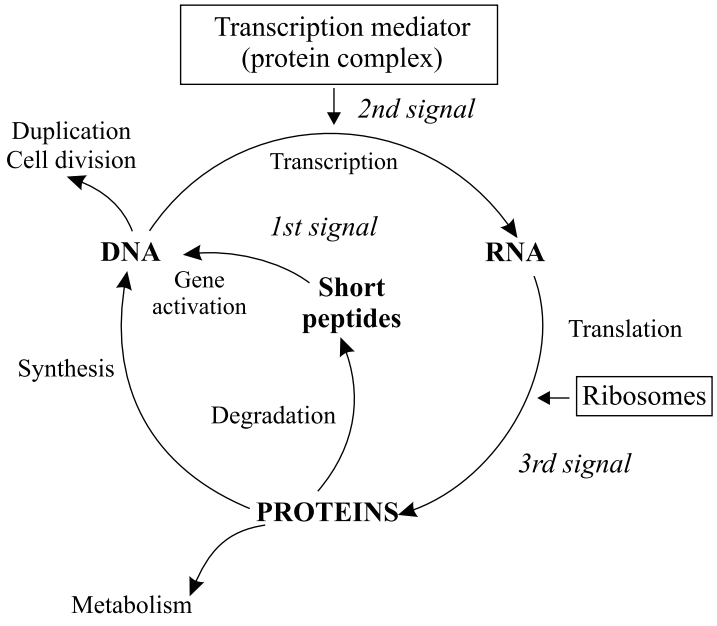

Fig. 2. Role of short peptides in the DNA, RNA, and protein biosynthesis cycles.

sites makes these sites unavailable for DNA methyltransferases, as a result of which the promoter remains unmethylated, which is the key factor for activation of the majority of genes.

On the other hand, the studied peptides Ala-Glu-Asp-Gly, Glu-Asp-Arg, Ala-Glu-Asp-Leu, Lys-Glu-Asp-Gly, Ala-Glu-Asp-Arg, and Lys-Glu-Asp-Trp-NH_2 differently modulate the endonuclease effects on DNA hydrolysis and their effects on this process can be to a certain measure be mediated through histones. This fact is very important, because in cells the peptides first of all have to find sites available for interaction with DNA in chromatin and this availability can be largely determined by histones, including H1 histone. Different effects of peptides on the endonuclease effects can be due to different site specificity of the peptide binding to DNA and different site specificity of these enzymes activities.

Hence, short peptides recognize and interact with the specific DNA sequences and exhibit their biological activity depending on the pattern of DNA methylation. Specific (complementary) peptide-DNA interactions can epigenetically regulate the cellular genetic functions promoting homeostasis recovery and lifespan prolongation. It seems that these interactions played an important role at the earliest stages of life emergence and during evolution [9].

The data obtained in studies of the molecular genetic mechanisms of peptide effects suggest a new concept most amply reflecting the evolutional biological role of peptides in the organism. Peptides and DNA are two classes of biopolymers carrying information and exchanging it during initiation of gene transcription. The peptide-DNA interactions promote recovery of gene expression and structure, this providing genetic stability and normalization of age-specific metabolic disorders, preventing the development of age-associated diseases, and prolonging the lifespan to the species limit [10]. In addition, peptides can be regarded as information regulators of genetic stability. This leads to stabilization of the main physiological functions and inhibition of aging. This is particularly important considering the key role of the genetic system in the mechanisms of individual development and emergence of many diseases. Importantly, regulatory peptides are new generation drugs realizing physiological correction without side effects.

These data on the molecular genetic mechanisms of short peptides action confirm the modern trends of proteomics and pharmacogenomics development and demonstrate the new approaches to prevention of aging and age-associated disease development [7,10,15].

Creation of drugs based on short peptides resulted in development of a new technology, genoregulatory therapy. It is based on complex use of peptide regulators of genetic stability and cell metabolism, which leads to normalization of the main physiological functions, increase of the adaptation potential, and inhibition of aging. For example, use of short peptides in patients engaged in complex coordination work and suffering from disorders of circadian rhythms caused a significant increase of HSPA1A gene expression. This gene codes for the production of heat shock proteins with molecular weights of 70 kDa (HSP70) protecting the cells from hypoxic and stress injuries. Individual selection of peptide preparations with consideration for genetic characteristics of the patients promoted improvement of physical endurance and mental stability.

Further development of this trend will open new approaches to studies of the mechanisms of ontogeny. More ample knowledge of the molecular genetic mechanisms of peptide effects will lead to creation of new geroprotectors for prevention of rapid aging and age-associated diseases.

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