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BOOK OF ABSTRACTS

Effective current approaches in anti-aging medicine and gerontology

MAIN FUNDAMENTAL PRINCIPLES OF GEROPROTECTING ACTION OF SHORT BIOLOGICALLY ACTIVE PEPTIDES ARE GENOME REPROGRAMMING AND REARRANGEMENT OF DNA METHYLATION

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Relevance: Aging is associated with genome reprogramming and rearrangement of DNA methylation pattern that is a marker of biological aging (biological clock) corresponding well to chronological age. So far as short peptides increase lifespan and improve physiological functions of various organisms [1], the main goal of this work was to investigate the molecular mechanisms of their action in animal and plant cells. A special attention was paid to study of the short peptide influence on expression of various genes involved in regulation of cell differentiation and plant growth and development, in particular.

Methods: Gene expression was measured by the real-time polymerase chain reaction (PCR-RT). RNA purification was done using RNA Protect Cell Reagent and RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. RNA samples obtained were used to synthesize the first strand of complementary DNA using oligo(dT)18 (Sintol, Moscow) and reverse transcription kit – Omniscript RT Kit (Qiagen, Germany) according to the kit manufacturer's recommendations. Quantitative PCR with the fluorescent dye SYBR Green I was performed by means of QuantiFast SYBR Green PCR Kit (Qiagen, Germany) and thermocycler CFX96 Real-Time PCR Detection System (BioRad Laboratories, Inc., USA). Construction of oligonucleotide primers was performed by means of online service NCBI Primer-Blast. The oligonucleotides used were synthesized by Sintol (Moscow, Russia).

Results: Short peptides can penetrate into animal cell, its nucleus and nucleolus [2]. Therefore, in principle, on this way they may meet and interact with various components of cytoplasm and nucleus including DNA, different RNA, proteins and chromatin histones, in particular. In fact, peptides in vitro interact site specifically with deoxyribo- and ribooligonucleotides, DNA, H1 and core histones [2].

In the human bronchial epithelium cells the peptide bronchogen (Ala-Glu-Asp-Leu) activates synthesis of anti-inflammatory proteins and cell regeneration factors (CD79a, Ki67, Mcl-1, p53), regulates expression of genes coding for differentiation factors (FoxA1, FoxA2, Nkx2.1, SCGB1A1, SCGB3A2) and other functional proteins (MUC4, MUC5AC, SftpA1), it changes the CpG

methylation status of promoters in respective genes [3]. Peptide pancragen (Lys-Glu-Asp-Trp) modulates transcription of genes coding for cell differentiation factors in the carcinoma cell culture from human pancreas. In particular, pancragen increased expression of differentiation factor Pax6 and others in aging cells of human pancreas. It induces the m^5CpG demethylation of Pax6 gene promoter.

Thus, short peptides modulate DNA methylation and, therefore, they may control all genetic functions including transcription, DNA replication and repair. The peptide activation of transcription is often associated with m^5CpG demethylation of gene promoters. We have suggested the most probable mechanism of such peptide regulation of transcription [4]: the peptide binding to gene promoter seems to protect CG or CNG site against methylation and these sites are left to be unmethylated that is crucial for activation of many genes. Specific peptide binding with histones may influence the various enzymatic histone modifications, and it can be another control mechanism of genetic functions. Specific peptide binding with miRNA or siRNA may be involved in control of the RNA-directed DNA methylations and gene silencing by RNA. Anyway, short peptides may modulate practically all known epigenetic mechanisms (DNA methylation, histone code, RNA gene silencing). Therefore, the peptide regulatory functions seem to have mainly epigenetic nature and signaling character.

Short biologically active peptides epitalon (Ala-Glu-Asp-Gly), bronchogen (Ala-Glu-Asp-Leu) and vilon (Lys-Glu) in concentrations 10^{-7}- 10^{-8} M influence essentially growth, development and differentiation of tobacco (Nicotiana tabacum) callus cultures [5]. Bronchogen significantly stimulates root growth in tobacco seedlings and makes them to be tolerant to grow in the presence of relatively high salt concentrations. In tobacco cells the peptides investigated modulate expression of many genes including genes responsible for tissue formation and cell differentiation. These peptides differently modulate expression of the CLE family genes coding for known endogenous regulatory peptides, the KNOX1 genes (transcription factor genes) and GRF (growth regulatory factor) genes [5]. They may also stimulate expression of the SNF family genes that are responsible for chromatin remodeling and activation of the RNA-directed DNA methylation. As a matter of fact, the modulation profile of gene expression with peptides in tobacco callus tissue and seedlings is different. This agrees with tissue specific action of peptides in animals [1]. Peptides studied may be related to a new generation of plant growth regulators. Such short peptides acting gene specifically both in animal and plant cells seem to be evolutionally early ones and common for eukaryotes, in general.

Conclusion: Short peptides represent an efficient signaling system of epigenetic control of cell physiology. They control all genetic processes. The

mechanisms of their action seem to be common for animals and plants. Short peptides are able to bind with DNA, histones, RNA, and they seem to be involved in chromatin remodeling. Anyway, they are involved in the genome reprogramming and rearrangement of DNA methylation. Peptides investigated belong to a new generation of plant growth regulators. The further investigation of peptide interactions with DNA, various RNA and chromatin, in particular, is very important for deciphering of mechanisms of gene functioning, cell differentiation and evolution. The search for and the design of new short biologically active peptides is a key promising way to the origin and production of a new generation of drugs that are gene addressed and strongly needed to prevent premature aging, to treat cancer and other diseases.

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