BIOGERONTOLOGY

Effects of Short Peptides on Lymphocyte Chromatin in Senile Subjects

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Effects of synthetic short peptides (Vilon, Epithalon, Livagen, Prostamax, and Cortagen) on activity of ribosome genes, parameters of common heterochromatin melting, polymorphism of structural heterochromatin (C segments) of chromosomes 1, 9, and 16, and variability of facultative heterochromatin were studied in leukocytes of subjects aged 75-88 years. All the studied peptides induced activation of ribosome genes, decondensation of densely packed chromatin fibrils, and release of genes repressed as a result of age-specific condensation of the cellular euchromatin regions (deheterochromatinization of facultative chromatin). Treatment with Epithalon, Livagen, and Prostamax led to decondensation of chromosome 1 pericentromeric structural chromatin, while Epithalon and Livagen treatment led to changes in chromosome 9 as well. Hence, short peptides activate heterochromatin and heterochromatinized regions of cell chromosomes in senile subjects.

Key Words: *short peptides; aging; heterochromatinization; chromatin activation*

Primary mechanisms of aging are related to changes in genetic processes leading to dysfunctions and inevitable age-specific diseases. The fact of age-associated impairment of the peptide bioregulator production in cells and changed sensitivity of target cells to these molecules is principally important, this making investigation of molecular genetic mechanisms of peptide effects during aging particularly important.

The potential of peptide bioregulators manifests by stimulation of immune and reparative processes and reduction of the probability of disease and accelerated aging; they are widely used in medical practice [10]. Peptide bioregulators induce metabolic changes controlled through gene regulation mechanisms realized in chromatin domains ("functional units" of heredity). Functional structural domains regulate the inter-

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related processes: genetic activity, time of reproduction, and degree of chromosome condensation.

Structural condensation of chromosomes closely correlates with functional heterogeneity. Compactly condensed heterochromatin, heterochromatinized (condensed eu- and heterochromatin) chromosome regions are genetically inactivated and their replication is delayed. Decondensed (euchromatin) chromatin regions actively function. An obligatory condition for transcription activity of genes is active chromatin [13]. Aging is associated with intense heterochromatinization paralleled by inactivation of previously active genes [12]. Heterochromatinization of chromosome regions is explained by lability of the cycle and is a reversible process [4].

We studied the effects of short peptides on the chromatin domain system in cultured lymphocytes from senile subjects, particularly their effects on chromatin reactivation — deheterochromatinization of heterochromatin satellite regions (activity of synthetic processes of ribosome genes), common, structural (vari-

ability of C-blocks of chromosomes 1, 9, and 16), and facultative chromatin.

MATERIALS AND METHODS

The study was carried out on chromosomes of 150 PHA-stimulated and 40 PHA-nonstimulated cultures of lymphocytes from 95 healthy individuals aged 75-88 years (control: 25 donors aged 20-40 years). Two cell cultures from each individual were studied: intact and stimulated with Vilon (Lys-Glu), Epithalon (Ala-Glu-Asp-Gly), Livagen (Lys-Glu-Asp-Ala), Prostamax (Lys-Glu-Asp-Pro), and Cortagen (Ala-Glu-Asp-Pro) for comparing the results of experiment with the control from the same individual. Thirty to 50 metaphase plates of intact and peptide-treated lymphocyte cultures (10 cultures in each group) were analyzed for each parameter. Studies by differential scanning microcalorimetry were carried out on 40 (20 intact and 20 peptide treated) PHA-nonstimulated 22-h cell cultures.

Peptide preparations were used in concentrations corresponding to their therapeutic doses. The final concentration of Epithalon and Livagen was 0.005 μg/ml, that of Vilon, Prostamax, and Cortagen 0.01 μg/ml [3,4].

Activity of ribosomal genes in intact and peptide-treated cultures was evaluated using Ag-binding and quantitation of associations of nucleolar organizer regions (NOR) of acrocentric chromosomes. Changes in activity were evaluated by comparing two binomial totalities.

Differential scanning microcalorimetry is based on different thermostability of different chromatin fractions. The sensitivity of this method is 10^7 cal/min, temperature interval 20-150ºC, rate of heating 35ºC/h, and volume of measuring cell 0.3 ml. The denaturing process was characterized by clearly reproducible heat absorption peaks [5].

Polymorphism of structural C-heterochromatin of chromosomes 1, 9, and 16 was evaluated by the method described previously [8]. Comparative analysis of C-stained chromosomes in intact and peptide-treated cultures was carried out using a standard classification, according to which the size of C-segments of chromosomes 1, 9, 16 was compared to the short arm of chromosome 16 and the results were classified into 5 categories (a, b, c, d, e). The χ^2 was estimated using Zax formula.

The variability of facultative heterochromatin was evaluated by the incidence of sister chromatid exchanges (SCE) in intact and peptide-treated lymphocytes. The cells were incubated during 2 replication cycles with 5-bromodeoxyuridine in a final concentration of 7.7 μg/ml. Differential staining of sister chromatids was carried out without fluorochromes [11]. The parameters of intact and peptide-induced cell cultures were compared using Student's *t* test.

RESULTS

In situ hybridization and differential Ag staining showed that human ribosome genes involved in protein synthesis are located in the satellite regions of acrocentric chromosomes (in NOR) [9]. Satellite regions participating in the nucleolus formation are predominantly heterochromatic areas.

The capacity of acrocentric chromosomes to form associations is determined by the presence of two chromatid satellite regions. Chromosomes are considered associated, if a pair of their satellite regions is connected. Associative capacity of satellite regions positively correlates with the intensity of Ag staining, which, in turn, is determined by activity of ribosome genes. One should remember that the absence of satellite sequence (because of condensation) attests to activation of ribosome genes [15]. The incidence of asso-

TABLE 1. Peptide Effects on Lymphocyte Chromatin in Senile Subjects

Experiment variant	Associating acrocentric chromosomes per cell	Deheterochromati-nization of facultative heterochroma-tin (SCE) per cell	Total heterochro-matin	Structural heterochromatin (C-blocks)		
				chromo-some 1	chromo-some 9	chromo-some 16
Control I (20-40 years)	1.33±0.06	7.7±0.4	Ss	Ss	Ss	Ss
Control II (75-88 years)	1.17±0.05*	5.9±0.2*	HC	HC	Ss	Ss
Vilon	2.39±0.11+	9.9±0.6+	DC	HC	Ss	Ss
Epithalon	2.32±0.12+	8.4±0.5+	DC	DC	DC	Ss
Livagen	2.49±0.14+	9.2±0.4+	DC	DC	DC	Ss
Prostamax	2.50±0.15+	12.0±0.8+	DC	DC	Ss	Ss
Cortagen	2.20±0.70+	10.1±0.7+	DC	HC	Ss	Ss

Note. Ss: stable status; HC: heterochromatinization; DC: deheterochromatinization. *$p<0.05$ compared to control I, +$p<0.001$ compared to control II.

dQ/dT, J/g×K
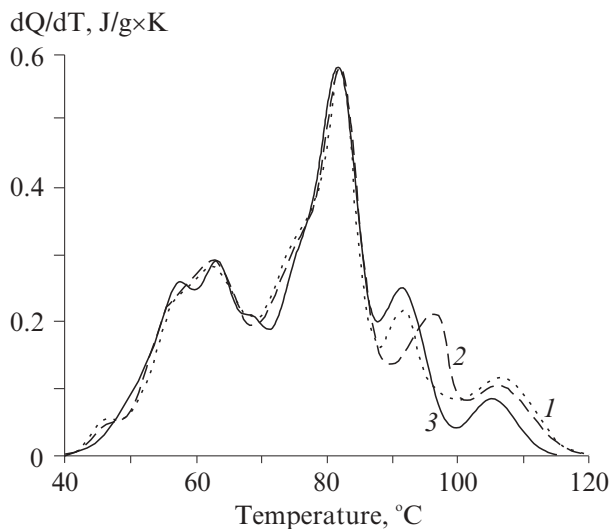
Fig. 1. Calorimetric curves of chromatin denaturation processes in senile subjects. *1*) intact lymphocytes of young (26-35 years) subjects; *2*) intact lymphocytes of senile (76-81 years) subjects; *3*) Vilon-treated lymphocytes of senile (76-81 years) subjects. Endotherm areas: I) 80.3°C; II) 95°C; III) 106.2°C.

ciations formed by silver-stained fragments of acrocentrics and the number of Ag-positive NOR per cell considerably decreased in senile subjects (because of heterochromatinization of satellite regions) in comparison with medium-aged subjects [12].

Analysis of the incidence of Ag-positive NOR showed different argentophilia of certain acrocentrics in the control and peptide-treated cell cultures (Table 1). The number of Ag-positive NOR of associative acrocentrics per cell in peptide-treated cultures was significantly higher in comparison with the control cultures derived from senile subjects. It is noteworthy that all 5 peptides promoted a steady increase in the incidence of DD-, DG-, and GG-type associations.

These results are in line with published data. It was reported, for example, that hormones and some growth factors promoted deheterochromatinization (decondensation) of chromosomes, in senile subjects as well, which resulted in increased transcription activity of the nucleolus-forming regions [2,4].

Increased number of Ag-positive NOR and high incidence of associations of acrocentric chromosomes in lymphocyte cultures treated with peptides indicates deheterochromatinization of the satellite regions, causing activation of ribosomal genes during aging. It is noteworthy that the expression of ribosomal genes is regulated by ATRX gene (member of SNF2-helicase/ATPase family) through modulation of the structure of satellite regions in acrocentric chromosomes [7].

Membranes, cytoplasmic structures, and nuclear proteins are denatured at 40-70°C, while chromatin is denatured at higher temperatures (74-107°C) [5,6]. The curves reflecting heat absorption by cultured lym-

phocytes without PHA stimulation and in peptide-treated cultures were characterized by intricate profiles of independent peaks. Endotherms I, II, and III corresponded to chromatin denaturing. Peptides added to lymphocyte culture caused changes in the profiles of heat absorption curves. For example, addition of Vilon (Fig. 1) shifted endotherms II and III towards lower temperatures by 2.9 and 1.0°C. Moreover, heat redistribution between transition stages II, III was observed: heat increased at stage II and decreased at stage III. Proceeding from published data, we hypothesized that the transition stage (endotherm) II was associated with uncoiling of 10- and 30-nm fibrils, while transition stage III was associated with uncoiling of loops consisting of 30-nm fibrils fixed to the nuclear matrix. These results indicate that Vilon causes heat redistribution between stages II and III, which is due to partial despiralization of loops to the level of 30-nm fibrils.

A similar picture was observed in the analysis of heat absorption curves after treatment with Epithalon, Livagen, Prostamax, and Cortagen, *i. e.* endotherms II and III were also shifted towards lower temperatures.

Hence, peptides Vilon, Epithalon, Livagen, Prostamax, and Cortagen unfold higher levels of chromatin organization in lymphocytes of senile subjects, deheterochromatinizint total (facultative and structural) heterochromatin.

The results reflecting variability in the incidence of large (d and e) and small variants (a and b) of C-segments in chromosomes 1, 9, and 16 are heterogeneous for the tested peptides (Table 1). Chromosomes 1 and 9 were heteromorphic under the effects Epithalon and Livagen, while Prostamax had such an effect only on chromosome 1; the degree of heteromorphism (decrease of the size of large blocks) was statistically significant for these chromosomes. After treatment with Vilon and Cortagen large and small variants of C-segments were recorded in chromosomes 1, 9, and 16 with about the same frequency as in intact cells.

Chromosomes 1 and 9 are characterized by pronounced variability of the absolute and relative sizes of C-heterochromatin in some diseases and during exposure to chemicals, while the distribution of heterochromatin C-segment variants in chromosome 16 remains stable [4,14]. In addition, elongation of C-segments of chromosome 1 (heterochromatinization of pericentromeric heterochromatin) was noted in senile subjects [11].

Our results suggest that peptide treatment decreased the size of C-blocks in chromosomes 1 (Epithalon, Livagen, and Prostamax) and 9 (Epithalon, Livagen), which indicates selective capacity of these drugs to induce decondensation (deheterochromatinization) of structural heterochromatin in cells of senile subjects.

The incidence of SCE is usually considered as an indicator of the reaction of cell homeostasis to endogenous and exogenous factors. This parameter can exhibit appreciable variability in different individuals, and therefore individual controls were used in testing of each substance (Table 1).

All studied peptides increased the incidence of SCE in cells from senile subjects (Table 1); the differences between the control and experimental values were significant for all variants.

The effects of the test bioregulators on the incidence of SCE varied for different groups of chromosomes. For example, Vilon and Livagen increased the number of exchanges for chromosome groups A, B, C, D, E, and G; Prostamax was active towards groups A, B, C, D, and G; Epithalon increased the number of exchanges for chromosome groups A, C, D, and G, and Cortagen for groups A, C, and D. All these changes were significant. These data attest to a selective effect of each bioregulator on certain chromosomes.

According to published data, no exchange processes are observed in heterochromatin and heterochromatinized regions of chromosomes [1], that is, SCE form mainly in euchromatin regions. Therefore, the detected increase of SCE values in lymphocytes of senile subjects is due to decondensation of the chromosomal heterochromatinized euchromatin regions under the effects of the studied peptides.

Hence, short peptides (Vilon, Epithalon, Livagen, Prostamax, and Cortagen) promote a "release" of genes repressed because of heterochromatinization of chromosomal euchromatin regions, associated with aging.

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