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Peptides and CCL11 and HMGB1 as Molecular Markers of Aging: Literature Review and Own Data

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Abstract—Cytokines CCL11 (eotaxin) and *HMGB*1 (alarmin 1) are molecular markers of ageing and neurological, cardiovascular, and immune diseases. Created at the St. Petersburg Institute of Bioregulation and Gerontology, these short peptides are known to regulate gene expression and protein synthesis. They promote decreased mortality and deceleration of pathology in the elderly. The review suggests that the dipeptide vilon (Lys-Glu) and tetrapeptide epitalon (Ala-Glu-Asp-Gly) are involved in regulation of the CCL11 and HMGB1 genes as activators of their expression. The geroprotective effect of vilon and epitalon is probably due to the suppression of these genes.

Keywords: *CCL*11, *HMGB*1, short peptides, aging
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In September 2011, *Nature* published a sensational article by a large team at Stanford University's School of Medicine headed by Professor Tony Wyss-Coray [52]. The authors reported that the blood of old mice contained substances that caused changes in the brains of young animals typical of the brains of aged animals. These substances induce apoptosis of brain neurons, and their content increases with age.

The authors used the parabiosis method by creating shared blood circulation between old and young mice. In this case, they observed a threefold increase in the number of neurons in the dentate gyrus of the brains of old animals. Injection of plasma of old animals into young mice led to the same negative changes in their dentate gyrus as if they would have a shared blood circulation. After this injection, young mice did worse in solving spatial navigation tasks, which indicated dysfunction of the hippocampus. In addition, cognitive processes in these mice were also impaired. The authors analyzed 66 different signal proteins of the immune system in mouse blood to reveal specific factors associated with aging and cell death. The concentration of six out of these proteins was increased in both old and young animals with shared blood circulation. One of them was chemokine CCL11 (eotaxin), which caused a decrease in the number of neurons in the dentate gyrus of young animals.

The study of a group of people aged 20–90 years showed that the chemokine CCL11 concentration in the blood and cerebrospinal fluid increased with age [39]. It was found in another study that expression of CCL11, gamma interferon (IFN-γ), chemotaxis proteins (MIG, IP10), and tumor necrosis factor receptor alpha (RTNF-α) in blood increased in people aged 40–80 years in comparison with young people [47]. At the same time, there was a significant decrease in the concentration of the main epidermal growth factor (EGF) and its receptor (REGF) in the older age group [47]. It has been assumed that the mediator in the development of nerve cell dysfunction in older people and animals injected with CCL11 is the transforming growth factor beta (TGF-β), the content of which decreases with age [40].

The CCL11 protein belongs to the CC chemokines; it is synthesized mostly by macrophages and is the chemotaxis factor of eosinophils. The gene encoding chemokine CCL11 is located on chromosome 17. It has been found that the CCL11 concentration is increased in blood and cerebrospinal fluid in patients with schizophrenia [27] but unchanged in patients with Parkinson's disease [45]. The inflammation processes are known to be the same for sterile injury and infection [6]. This cascade of reactions occurs with the involvement of components of tissue fluid, lymph, plasma, leukocytes, thrombocytes, endothelium, and connective tissue cells. The action of an irritant leads to the formation of factors (so called alarmins) in inflammatory cells, which transmit a danger signal to other cells. Alarmins transmit the information resembling signals, which come from endogenous sources of damage, DAMP (damage-associated molecular pattern molecules) [43].

Thirteen alarmins have been found to date; however, many molecules of mono- and polynuclear

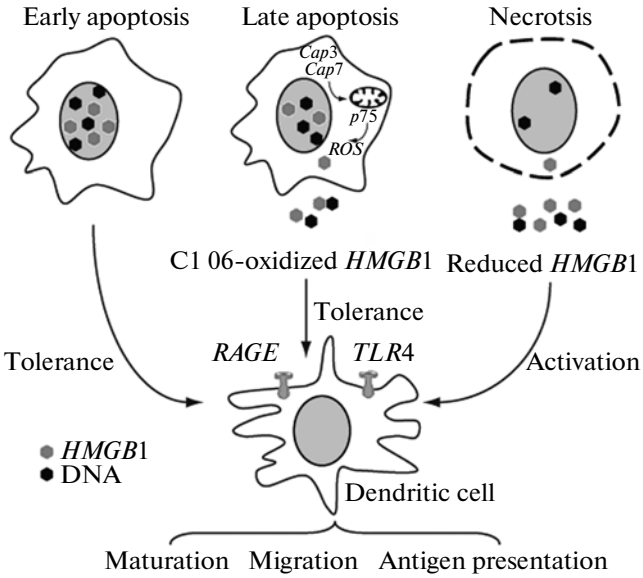

Fig. 1. @

phagocytes, eosinophils, endothelial and epithelial mast cells, and thrombocytes correspond to the notion of alarmins. One of the main alarmins is nonhistone chromosomal cytokine, HMGB1 (high-mobility group box chromosomal protein 1), which was isolated for the first time in 1999 from calf thymus. The HMGB1 protein is predominantly contained in cell nuclei and functions as a DNA chaperon. A decrease in the HMGB1 level in cell nuclei leads to a decrease in both the concentration of histones and the number of nucleosomes. This disrupts the protection of DNA and decreases the expression of 10% of genes. A reduction in the number of nucleosomes is accompanied by a change in the specific genome functions, which leads to a change in epigenetic regulation. The HMGB1 gene contains nine transcripts. GenBank now contains information on five promoter regions of this gene (NM 002128.4). The HMGB1 protein is released during cell necrosis and apoptosis and is secreted by macrophages as proinflammatory mediator [44]. The release of the HMGB1 protein during apoptosis is due to the nucleosome fragmentation of DNA, which is catalyzed by caspase-activated deoxyribonuclease (Fig. 1) [52].

Like proinflammtory cytokine, HMGB1 significantly increases the proliferation of cells with the $CD4^+$ and $CD8^+$ markers [47]. The HMGB1 protein interacts with the toll-like receptors TLR2, TLR4, and TLR9 [44, 46]. IL-1β and TNF-α cause a significant increase in the HMGB1 protein concentration and promote HMGB1 translocation from cell nucleus to cytoplasm [50].

It was found that the systemic inflammatory reaction leads to inhibition of transcription of the IL-1β and TNF-α genes. In this case, the NF-κB factor is stimulated, and the H3K9 histone is demethylated. Moreover, the HMGB1 and H1 histone nucleosomal proteins are necessary components of endotoxin-induced inhibition of TNF-α in human THP-1 promonocytes. At the same time, HMGB1 inhibits transcription and binds TNF-α, which minimizes the effect of the assemble in the promoter of the gene receptor [28].

It has now been established that free (extracellular) HMGB1 is involved in all phases of inflammation, from damage to tissue repair [42, 43]. It activates endothelial cells and their precursors, which enhances expression of the adhesive ICAM-1 and VCAM-1 molecules and the release of proinflammatory cytokines, which is accompanied by adhesion of monocytes/macrophages, neutrophils, and, probably, thrombocytes on the inflammatory endothelium [51].

The HMGB1 cytokine plays an extremely important role in sepsis. It is known that HMGB1 binds to DNA in cells and modulates the intracellular processes [51]. The appearance of HMGB1 outside a cell is a signal of distress, which indicates significant tissue damages. This is accompanied by increased blood clotting up to the development of disseminated intravascular blood coagulation. The presence of HMGB1 in blood circulation in sepsis indicates an extremely poor prognosis. This cytokine appears in human and mouse serum 8–32 h after administration of lipopolysaccharide [38]. Injection of the recombinant HMGB1 protein reproduces many features typical of sepsis including fever, disturbance of intestinal barrier function, respiratory distress syndrome, and multiple organ failure. The latter is due not only to the ability of HMGB1 to bind heparin and proteoglycans, but also to decrease the content of thrombomodulin on the activated endothelium [18].

The HMGB1 protein has been proven as an inductor of fatty degeneration of the arterial wall, which supports chronic inflammation in atherosclerotic plaques. In this case, the cellular matrix is destroyed, which is accompanied by necrotic changes in the endothelium. The involvement of HMGB1 in the atherosclerotic process may be associated with stimulation of endotheliocytes, producers of proinflammatory cytokines, which enhance the expression of adhesive molecules and involve macrophages at the inflammatory focus (Fig. 2). The HMGB1 protein accumulates not only in the endothelial, but also in smooth muscle and foam cells. Blockage of the HMGB1 protein with antibodies prevents the development of atherosclerosis and reduces the intensity of ischemic stroke [51]. HMGB1 inhibits collagen synthesis with fibroblasts. This effect is probably caused by RAGE (receptor for advanced glycation and products) activation. At the same time, senescent fibroblasts secrete the oxidized HMGB1 protein, which stimulates the secretion of proinflammatory cytokines, including IL-6, by activating the TLR-4 receptor [25].

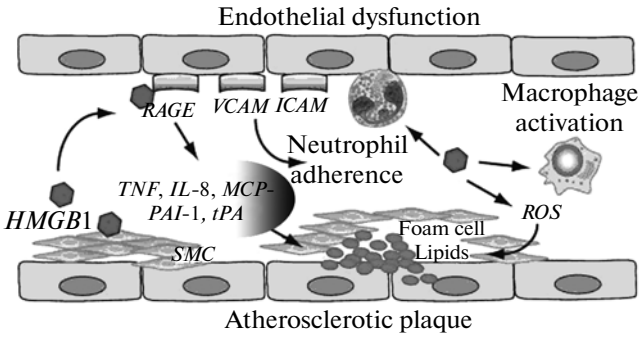

Fig. 2. @

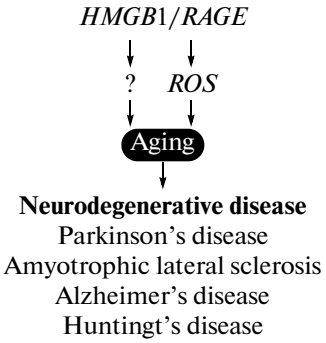

Fig. 3. @

With aging, there is a redistribution of the HMGB1 protein between the nucleus and intercellular environment. In contrast to secretion, this reaction requires the presence of the *p*-53 protein, which suppresses the tumor process. Blockage of the HMGB1 protein with antibodies or disabling of TLR-4 leads to a sharp decrease in IL-6 secretion, while the introduction of exogenous HMGB1 is accompanied by NF-κB stimulation, which restores the IL-6 level in cells exhausted by alarmins. Perhaps, the aging process, which is simultaneously the inflammatory reaction, is largely determined by mutual regulation of HMGB1 and *p53* [25]. Special attention should be paid to the fact that extracellular HMGB1 is now considered a molecular target in treating inflammatory diseases and some cancers [47–50]. Another important fact is that HMGB1 binds thrombomodulin, which, along with enhancement of tissue factor expression, contributes to the development of hypercoagulability and thrombotic complications [18]. All this cannot but affect the process of premature aging and intellectual functions.

The HMGB1 protein plays an important role in the pathogenesis of diseases, such as rheumatoid arthritis, graft rejection, and the development of ischemia and autoimmune liver injury [24]. It was found that β-amyloid accumulates in the brain during Alzheimer's disease, which is caused by the activation of microglia. Two forms of this protein are known, Aβ40 and Aβ42. Microglia are found to be able to phagocytose the Aβ42 and HMGB1 proteins. The introduction of alarmin 1 inhibits the degradation of Aβ40 in microglia. Moreover, microglia are activated in the brain of Alzheimer's patients and adhere the Aβ40 and extracellular HMGB1 proteins, which is accompanied by progression of pathology. Consequently, HMGB1 is one of the pathogenic factors of Alzheimer's disease (Fig. 3). It is assumed that the inhibition of HMGB1 synthesis may improve the condition of such patients [49]. Thus, the HMGB1 cytokine plays an important role in the aging process and development of age-related diseases.

Researchers at the St. Petersburg Institute of Bioregulation and Gerontology synthesized the geroprotective peptides vilon (Lys-Glu) and epitalon (Ala-Glu-Asp-Gly) [2, 8–13, 35]. These peptides were found to be capable of activating cognitive function in aged animals and the elderly [11, 13, 15, 34, 35]. In a model of accelerated aging caused by γ radiation, vilon modulates the immune response and activates postradiation recovery of the thymus and spleen. In addition, vilon stimulates the proliferation and differentiation of thymocytes and the migration of leukocytes [10, 11].

It was shown in in vivo experiments that vilon stimulated innate and adaptive immunity [13]. Vilon also stimulated the functional activity of *T* and *B* lymphocytes and the production of the proinflammatory IL-1α, IL-1β, IL-8, and TNF-α cytokines in patients with secondary immunodeficiency. Vilon decreased IL-8 concentration and increased INF-γ synthesis in a lymphocyte culture [11]. Single intranasal administration of vilon led to an increase in IL-2 mRNA gene expression in the LNA cells of the hypothalamus, which indicated its ability to regulate gene expression [5]. It has been demonstrated that the GCAG and CGTC sequences, which are potential binding sites of vilon, may present in promoter regions of the IL-2, IL-5, IL-6, IL-17A, TNF-α, and INF-α genes [7, 13].

The introduction of vilon in CBA mice helped to increase maximum life span and reduced the incidence of spontaneous tumors by a factor of 1.5. Incidences of adenoma of the lungs and breast adenocarcinoma were reduced in animals under the action of vilon by a factor of 2.5. The number of mice that survived up to 23 months after treatment with vilon was 2.6 times higher than in the control group. In this case, the maximum life span under the action of vilon increased by almost two months [10, 11, 15, 19, 31].

In an experiment with VES Drosophila melanogaster, epitalon slowed age-related changes in the reproductive and immune systems. Epitalon increased life expectancy by 17% and reduced the rate of aging of an organism by a factor of 2. Moreover, epitalon led to a decrease in the concentration of conjugated hydroperoxides and ketodienes in tissues of female flies and leveled sex differences in the content of lipid peroxidation products (the LPO level in female flies was significantly higher than that in male flies in the control

Table 1. Possible binding sites of vilon and epitalon in promoter region of *CCL*11 gene

Gene	Regulatory region of gene from 499 to 100 np (cDNA 5'-3')
CCL-11 (*ENSG*00000172156)	*GAGATGCAACTATGT**GCAG**GG**CTGC**TGAGCTCTCT**CTGC**ATCTGGGTGGGAGCTAAT GGAAGTTTTGGGGCTCCTTCCTGGTCTCCAAAATCCTCAAGACCACCATGTGAACACA GGAATCAAGGAAGGTTCTTAGATCGACTCATCCCCCAGGCCTTTGGTTTCCTTGCTCCTT TCCCCAACTACAGGTGTTTC**ATTTC**AACTCATCCCCTAGGGCCTTGGTTTTCTGCTCTCT TCCCCCACTACAGATGTTTAACTTC**ATTTC**ATAACCACATATTCCCCTCCTTTTCCAAGGCA AGATCCAGATGGATTAAAAAATGTACCAAGTCCCTCCTACTAGCTTGCCTCTCTTCTGTT **CTGC**TTGACTTCCTAGGATCTGGAATCTGGTCAGCAATCAGGAATCCCTTCATCGTGACC CCCGCATGGGCAAAGGCTTCCCTGGAATCTCCCACACTGT**CTGC**TCCCTATAAAAG **GCAGGCAG**ATGGGCCAGAGGA**GCAG**AGAGGCTGAGACCAACCCAGAAACCACCACC TCTCACGCCAAAGCTCACACCTTCAGCCTCCAACATGAAGGTCTCC**GCAG**CACTTCT*

Here and in Table 2, the binding sites of epitalon and vilon are in bold and in bold with underlining, respectively.

group) [33]. Numerous experiments showed that epitalon had a geroprotective effect and increased the lifetime of animals [1, 9, 13, 19–26, 29–34].

When kept under conditions of constant illumination, male and female rats showed accelerated aging in comparison with rats kept in the ordinary light mode. In addition, Vinogradova et al. [4] observed the appearance of spontaneous tumors and a decrease in life span. The use of epitalon reduced the adverse impact of constant lighting, improved the homeostasis parameters, and slowed the aging process. Epitalon inhibited the development of spontaneous tumors. Under impaired light conditions, epitalon demonstrated more pronounced geroprotective and anticancer properties than melatonin [4]. It should be noted that epitalon significantly reduces the number and intensity of spontaneous tumors in SHR mice and inhibits the growth of rat tumors induced by 1,2-dimethylhydrazone [37].

The in vitro experiments showed that the studied peptides are able to penetrate cells and bind to DNA [26]. Using array technology, it was found that vilon and epitalon regulated the expression of genes whose functions corresponded to different cell systems [5]. Thus, these peptides have selective properties with respect to binding sites on promoter regions of specific genes. It is assumed that amino acid residues of short peptides form a network of hydrogen bonds with nitrogenous bases in a large groove of the DNA double helix. Earlier, Khavinson et al. [36] proposed models of the specific binding of epitalon to ATTTC, ATTTG, and CTTTC sequences and vilon to the GCAG sequence. 3D models of the interaction of the Lys–Glu and Ala–Glu–Asp–Gly peptides with the GCAG and ATTTC regions of DNA, respectively, were designed from an analysis of the literature data on the interaction of different proteins with DNA. Analysis of the main parameters of molecular mechanics (the number of hydrogen bonds, hydrophobic and electrostatic interactions, energy minimization of the DNA-peptide complex, etc.) made it possible validate the earlier proposed quality models and to identify the most energetically favorable DNA–peptide complexes [37].

The data suggest that the geroprotective effect of vilon and epitalon may be due to their suppressive influence on the CCL11 and HMGB1 genes.

The binding sites of vilon and epitalon in the CCL11 gene were studied using data on gene nucleotide sequences. The promoter regions of the CCL11 and HMGB1 genes were found using the Ensemble Genome Browser system under the numbers ENSG00000172156 and ENSG00000189403, respectively.

Nine supposed binding sites for vilon to the GCAG and complementary CTGC sequences and two sites for epitalon to the ATTTC and ATTG and complementary TAAG and TAAC sequences were found in the promoter region of the CCL11 gene (Table 1).

It is of considerable interest to identify the binding sites in the promoter regions of the HMGB-1 gene for vilon and epitalon (Table 2). Promoters 1 and 3 of the HMGB-1 gene contain the binding sites for vilon (ten and five, respectively) and no binding sites for epitalon. Promoters 2 and 4 of the HMGB-1 gene contain five and eight binding sites, respectively, for vilon and one binding site each for epitalon. One binding site for vilon and four sites for epitalon were found in promoter 5 of the HMGB-1 gene, with the GCAG, CGTC, and GACG sequences being binding sites for vilon and the ATTTG and TAAAG sequences being binding sites for epitalon.

These data suggest that vilon and epitalon may regulate the HMGB-1 gene, which plays an important role in the development of Alzheimer's and autoimmune diseases. The number of binding sites for vilon

Table 2. Possible binding sites of vilon and epitalon in promoter region of *HMBG*1 gene

Gene, *Homo Sapiens*	Regulatory region of gene from 499 to 100 np (cDNA 5'-3')
*HMGB*1, high mobility group box 1 (*ENSG*00000189403)	***Promoter 1. HMGB1_1/NC_000013 31191625..31192224 1-*** *GGAAAGAAACCCCTCCCTCTTCTCCCTTACCTGCCGCGGGCACTCCCCTTCTTGGTACCGGGTCGATC* *GGAACTCCTGTTCCAGCTTGATCTCCACCCTAGTTGCAACGTTCAACCCACGTTCCCCTCGGACTGCTC* *CTCCCCCACTCG**CGTC**TCCACTAGGAAGGCGGCTTCCGGCTTGAGTCCGCGGCAAAAGAGTCCTCCTT* *CCTGCTGCACGCTGGGCCTGAAAG**GACG**GTGGCGTGGCGGGGAAGGTGAA**GACG**TGAGCACTTCCG* *GTCGCCCTCC**GCAG**AGGCGTGGCTGTCCGCCCTGTGGCC**GCAGACGCAG**TTGCGACTGCGGC**GACG*** *AGGAGGGGCGGGGCCGGTGGCTGCTGAGCCCGCCATGTGTGAGTGGCTGGGTTTGGGGAGGC**GACG*** *TTTCTGGAAGCTGCTGGAAAGCGCCCGAGTGGCGGAGGTGGCGCCAGCGGCCAGGGGGTGGGGCGT* *GATTAAAACTAGTGCGCGGCTTTCTGCGGAGGGATTACGCT**GACG**AAAGAGACCTGCTTGCG**CGTC**GCT* *GTTCCGTGGTCCGCGCGAGCGTGGTCGGGAGCCGCTGGTTCCTGGGGTGACCCGCGGAGGT*
	Promoter 2. HMGB1_2/NC_000013 31039974..31040573 1- *CCCTCAGCACCA**GCAG**AGACCCCAGTTTTCAGGGGACATGATCCCATAGTGTCGCCCTCACTTTTGAA* *GGGCCATTAAAAGCCTGGGGCCTCTTATCTCAACGGCTTTGGGCGTGAATGTGGGGCAAGAAGGGGGG* *GGGAGACCTGTGGGTGTCTCTTTTGCCTGAGGAGTTGGAGACACTTGTGGAAAAGTCAGGCCCTTTTC* *GCTCCGGCGGCCGCTCCGGTGTGGGGCTGGCTTGGGTTAGACACATGCACACATACACCATAGAGCTC* *TGCTTTCCCGTA**GCAG**CTGCTGCCTCTGCCTCTGCCTCTCCCGCCTCAGCCTCTTTGCCCGGCATACAC* *ACACATTCAG**ATTTG**CGCGCTGTTTCAATCCTTGAT**GACG**TGTCCCCGGAGACAGCCAATAGCAAACGG* *GCTCTGGTCAGGACAATGGGAGGTATCGGGCCAATGAGCGAGCCCCGTGAGTTGGCGGTAGCCAATAG* *GAGCCGCGCTGGCTGGAGAGTAATGTTACAGAGCGGAGAGAGTGAGGAGGCTG**CGTC**TGGCTCCCGC* *TCTCACAGCCATT**GCAG**TACATTGAGCTCCATAGAGACAGCACCGGGGCAAGTG*
	Promoter 3. HMGB1_3/NC_000013 31039278..31039877 1- *GCGCGGCGGCCGGATCCCCGCGGCCGGGAGCCGGCGGGTCAGGATCCACACAAAGGCAAATGAGGG* *GGGACCGTGGGGGGAACTGCGCACGGAGCGAGCCTCTGCCCGGGCGCCGGGAACGCTGCCCCGCG* *CCGGTGCCCCGGCCCTCAG**GCAG**CCTGAGGCGCCGGGAGCCCCGCGCCCCGCGAGTTTCCACCCCC* *GGCGG**CGTC**CGCGCTGACTGGCGCAAAAAAAAAAATTTTTTTTTTAATTAAAAAAATTTTGAACGTGTTTT* *GGGCCCTCGGGCCGGGCGTTCGGGCGGGCGGCGTGCGCGGAGCGCGGCCGGGGCGGCGGGGCCG* *GCGCGGCTCGGCGGCGGCGGCGGGAGG**GCAG**CGGCGGCGCTTCCCCGGGCTGCATTGGCCGCCGC* *C**GCAG**CGAGCCGGGCGCTGGCGGGGAGCGCGGCCAGCCGGGCGGGCGGCGGGGCGGGCGGGGC* *GCCGCGGCGGGCGAGGGCGGCGCGGGGGCCTGGGGGCG**GCAG**TGCGGGCCCGGCCGGCCTCGGC* *CCGGTCGCGGCGGCGGCGGCCGGGCGGCGGGGGGAGCGGCGCCGCTGCGCTCGCTGGAACAT* *GGCTGACTCGG*
	Promoter 4. HMGBl _4/NC_000013 31038365.31038964 1- *CCGCCGCGCCCGCACCCTCGCACTCACACACTCTCTCATACACACACACACACACACACACACAAAG* *GGAAGGAGCCATATTCTCGCTCGCGCTCGCCCTCGCGGCGGCGGCGGC**GCAG**GCGGAGAA**GACG**C**G*** ***CAG**CGGCCATTCCGTGCGCGCCGGCCCCGGCGGCCGCGGGCGGAGCCAGCCCCC**ATTTC**GAGCGG* *GGCTTCTCCCTGCGCCGAGCCTGACAAAATGGGGGCGGCGGCGGCGCGGGCCT**GCAG**GGCCTGCCG* *GGCGCACGTGGCGGCCTCGGGCCTGGGAGCCGGGCCG**CGTC**CTCTCTCCTCGGCCGCGCGGCCAC* *CGGCGAAGTTCTAGGGGCGGGGGGCTCGCCCCGC**GCAG**GAGTCACCCCAACTTTCACGGCTCCAAAA* *AATACTTCCCGAGTTGGGGGAGGGGGCCACCGAGCCACGA**GCAG**GAGTGGCTTTTGTCCCTCATCCTT* *GTTTACTCGGAGAAACTTCAGACCG**GACG**TGTTTAGTCAGAACAGAAATACATCTCAGGGCCAAACCGAT* *AGGAAACGAGGCTGCCTCGCGGTGGCACCGCCACCCCCCAACCGGGTTCCGAGCACCGGAGCTG*
	Promoter 5. HMGB1_5INC_000013 31037341.31037940 1- *TACTGTGTTAAATAACCAGTACTTTGGTTTTCATTCCCTTACTAAGTACTTTAAGGTCTTATATGTCATAATTT* *TATTGCTAACATCAAATATTTATTTTATTTTTTAGAAAAATAAC**TAAAC**ATGGGCAAAGGAGATCCTAAGAAG* *CCGAGAGGCAAAATGTCATCATATGCATTTTTTGTGCAAACTTGTCGGGAGGAGCATAAGAAGAAGCACC* *CAGATGCTTCAGTCAACTTCTCAGAGTTTTCTAAGAAGTGCTCAGAGAGGTGGAAGGTAAGAGGGCTTAA* *AACATGCTAACAAGGTAATTAAAAGACAGTTTCCAATTGAGGATGCAAAAAAAAGCCTAGTTGGCATTCTC* *GTAGTGG**GACG**CTATTACATAGCAAAAGACATTGGTTTTGAGGATAATTTACTTAAATGTTACAACT**TAAAC*** *TTACAAATAATTATTTTGTAGACCATGTCTGC**TAAAG**AGAAAGGAAA**ATTTG**AAGATATGGCAAAAGCGGA* *CAAGGCCCGTTATGAAAGAGAAATGAAAACCTATATCCCTCCCAAAGGGGAGACAAAAAAGAAGTTCAAG* *GATCCCAATGCACCCAAGAGGCCTCCGTG*

The first column contains the number of the gene in GenBank.

in HMGB-1 gene promoters significantly exceeds those for epitalon. Probably, vilon will yield a more intense effect on HMGB1 gene expression.

These data suggest that the geroprotective effect and enhancement of cognitive functions under the action of vilon are largely due to suppression of the CCL11 gene and, thereby, inhibition of synthesis of the CCL11 protein. The final answer to this question requires proof that the influence of vilon on the CCL11 gene is accompanied by increased DNA methylation and decreased histone acetylation.

However, there is another aspect to the subject. CCL11 is a potent chemoattractant not only for eosinophils but also for basophils, which makes it one of the main components of allergic reactions. Blockage of CCL11 by antibodies significantly improves the condition of animals infected by a respiratory virus. In addition, the concentration of IL-5, which is the main factor of proliferation and maturation of eosinophils, decreases. Moreover, CCL11 inhibits the action of $CD4^+$ lymphocytes [39].

These data indicate that allergic, infectious, inflammatory, and other diseases may be especially severe for elderly with poor innate and adaptive immunity and an increased CCL11 concentration [39]. The search for drugs aimed at reducing CCL11 concentration may be one of the leading problems of modern gerontology and geriatrics. It is possible that vilon may be one of these compounds.

Like IP10 (interferon gamma-inducible protein 10), CCL11 is a biomarker for the early stages of age-related macular degeneration [41]. It was found earlier that epitalon is the efficient retinoprotector for the treatment of age-related macular degeneration, retina pigment, and diabetic retinopathy in patients of older age groups [15]. At the molecular cellular level, epitalon stimulates differentiation of neurons in the retina and the pigment epithelium by regulating the synthesis of the Vsx1, Chx10, Pax6, Brn3, Math1, Prox1, and TTR transcription factors. The genes of these proteins were found to contain the ATTTC sequence complementary to epitalon [16].

Special attention should be paid to the presence of binding sites for vilon and epitalon in HMGB1 gene promoters. Taking into account our previous studies [2, 8–14, 35], which show that vilon and epitalon have a geroprotective effect and inhibit the development of cardiovascular and oncological diseases, we assume that both peptides can suppress the HMGB1 gene.

In conclusion, it should be noted that both vilon and epitalon, since they have affinity to the binding sites in the promoter regions of the CCL11 and HMGB1 genes, can epigenetically slow the development of aging processes [3, 17], significantly delay manifestation of atherosclerosis and associated cardiovascular diseases, and considerably improve cognitive functions in people of all ages.

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