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GDF11 Protein as a Geroprotector

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Abstract—GDF11 protein, the growth differentiation factor 11, which belongs to the TGF-β superfamily (transforming growth factor β), shows marked geroprotective effects on the cardiovascular and nervous systems. The cardioprotective and myoprotective effects of the GDF11 protein are associated with its regulation of several signaling molecules, including the MAPK–p38–mioglianin pathway. GDF11 neuroprotective action is associated with the regulation of proliferation and differentiation of brain neurons by means of changing the activity of the p57 (Kip2) and p27 (Kip1) transcription factors. GDF11 may be considered a potential target for geroprotector drugs, as was demonstrated in the case of the Glu-Asp-Arg peptide possessing similar neuroprotective and myoprotective properties as GDF11. For the Glu-Asp-Arg, Ala-Glu-Asp-Gly, and Lys-Glu peptides, binding sites were found in the promoter region of *GDF11*: the CCTGC, ATTTC, and GCAG motifs, respectively.

Keywords: growth differentiating factor 11, Glu-Asp-Arg peptide, geroprotector, neuroprotector, cardioprotector

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It is a well-known fact that it was quite a common practice in the past to infuse to athletes before important competitions with their own blood, which was prepared beforehand. The positive effect of such a procedure was generally accounted for by the resulting increase in the circulating blood volume and, as a consequence, a better oxygen supply to the tissues. But was it really the case?

The increased total volume of the circulating blood makes heart work harder while it is under a considerable load in athletes as a matter of fact. Moreover, under intense physical work conditions, the oxygenated hemoglobin dissociation curve shifts rightwards and downwards with an increase in the arterial-venous oxygen content difference, but the oxygen contained in the blood is still not completely released. This means that venous blood retains a relatively high reserve oxygen amount during intense physical work (Kolchinskaya, 1991). Ten days prior to competitions, up to 400 mL of blood is drawn from the sportsman and preserved. Bloodletting causes not only slight hypoxia but also activates the proliferation of the blood cells. In addition, it stimulates the activity of the sympathetic nervous, reticulo-endothelial, and immune systems. When blood is stored for 10 days, biologically active compounds are produced in it. The infusion of this stored blood on the day of competition improves the athlete's performance. Moreover, it is possible to administer simultaneously with blood vitamins, energizers, antihypoxants, and other biologically active compounds (Pottgiesser et al., 2011).

Apart from aerobic cyclic sports, autohemotransfusion as an efficient means of enhancing the organism's resistance to hypoxia may be useful in the case of summit ascents, deep diving, and in all other cases in which such resistance is required (Pottgiesser et al., 2011).

Let us recall the tragic story of the professor A.A. Bogdanov, the founder of the Institute for Hematology and Blood Transfusion. He set out a hypothesis that the transfusion of blood from the young into the elderly might lead to rejuvenation of the latter. At that time the four blood groups had already been identified and the principles of blood compatibility had been already known. Being a man in years at that time, A.A. Bogdanov made a decision to run the experiment on himself. From 1924 to 1928 he performed 11 blood transfusions, five of which involved blood volumes of 900 mL. He survived these operations without any problems and observed for himself quite satisfactory effects. Unfortunately, back then many of the presently known erythrocyte antigens had not been yet discovered, including the Rh factor; as they were not taken into account, multiple blood transfusions were rather dangerous. The next blood transfusion was to be

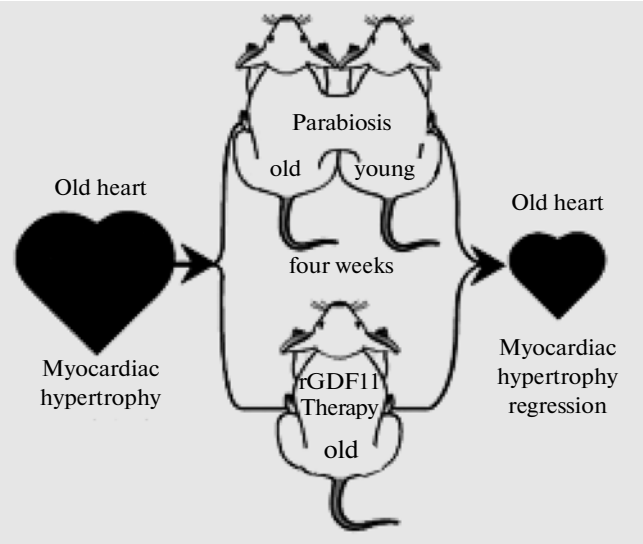

Fig. 1. Miocardiac hypertrophy regression in heterogenous parabiosis in mice (modified from Loffredo et al., 2013).

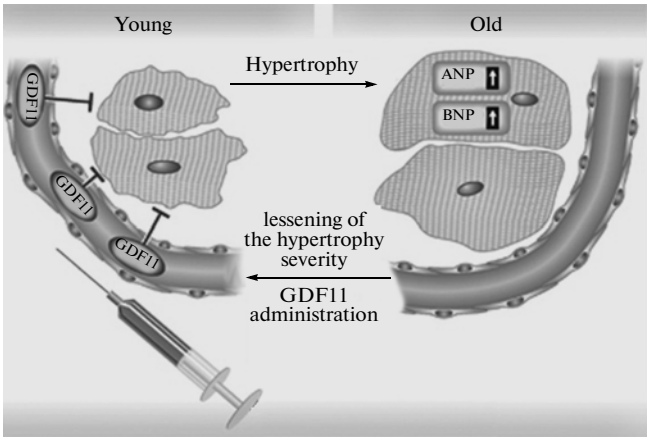

Fig. 2. Presumable mechanisms of GDF11 cardioprotective action in myocardiac hypertrophy (modified from Brack et al., 2013).

fatal for Alexander Alexandrovich (Donskov and Yagodinskii, 2006).

When heterochronic parabiosis was used to study the effects of the blood of young mice on elder mice (Loffredo et al., 2013) with cardiac hypertrophy (Fig. 1), four weeks after the start of the experiment disease regression was observed in elder mice. Also, a decrease in the size cardiomyocytes and higher cross-striation rate of the cells were noted. The age-related hypertrophy reversion was in no way associated with the animal's sex, improved hemodynamics, or parabiosis. The case was that some unknown factor had been transferred with the young mice blood to the elder mice (Fig. 1).

Subsequent studies demonstrated that this factor that circulates in the blood of the young mice was GDF11 (growth differentiation factor 11), which belongs to the TGF-β (transforming growth factor β) superfamily. Its content in the blood decreases with age. Infusing the young mice's blood to the elder mice reconstituted the GDF11 content, which caused a complete regression of the age-related heart hypertrophy. It was accompanied by the prolonged diastole time, which was facilitated by the cardiomyocytes relaxation. Similar results were obtained in the case of the intraperitoneal injection of the recombinant GDF11 protein into the old mice. Therefore, GDF11 may be used with therapeutic purposes to cure heart hypertophy in the elderly (Loffredo et al., 2013).

Studying the molecular mechanisms of the cardiac hypertrophy revealed that brain natriuretic peptide (BNP) and atrioventricular natriuretic peptide (ANP) are involved in this pathology development. On the basis of the obtained data, a concept (Brack, 2013), explaining the cardioprotective action of GDF11 in cardiac hypertrophy was proposed. When GDF11 synthesis is blocked, cell hypertrophy develops, leading to their accelerated aging (uppermost arrow in Fig. 2). In the case of hypertrophic changes, GDF11 administration leads to the restoration of the cell structure and functions.

The most intensive synthesis of GDF11 was observed in the spleen tissues (Loffredo et al., 2013) and heart intercalating disks of old mice. In this connection, the question arises as to whether the spleen is actually the source of GDF11 and why this factor, which is present in the heart, is not able to protect it from hypertrophy (Brack, 2013). This solution to this question (its important theoretical value notwithstanding) will open new prospects in developing therapeutic approaches to cure age-related heart hypertrophy.

In works performed by scientists from Harvard and Oxford, blood transfusion from young mice to the elder mice and the establishment of heterochronic parabiosis, which involves the joining of the circulation systems of young and old animals, enhanced locomotor activity, and improved cognitive abilities in the elder mice. Blood transfusion from one elder mouse to another elder mouse did not show any positive effects (Villeda et al., 2014). The observed phenomena were accounted for by the transfer of the GDF11 protein, the contents of which decreased with age, from the blood of young animals to elder animals.

GDF11 Protein Effects on Functional Activity of the Myocard and Skeletal Muscles in Aging

GDF11 protein was discovered 20 years ago. So far, its recombinant analog, rGDF11, was obtained. It is a disulfide-bound dimer with a molecular weight of 25KDa, with each polypeptide chain in the dimer containing 109 amino acid residues. rGDF11 has the same geroprotective effects as blood transfusion from young animals to the elder ones (Sinha et al., 2014).

Upon rGDF11 administration to the old mice, not only geroprotective effects but also an enhancement of the functional activity of skeletal muscles were observed (Sinha et al., 2014). The structural and functional properties of muscles improved in the old mice, together with the increase in their strength. At the functional level, old mice treated with rGDF11 showed a higher exercise tolerance; they were more tolerant to high lactate doses, and influenza virus infection was quite mild in them. rGDF considerably improved regenerative processes in the muscle tissue.

In vitro studies have added much to the understanding of the mechanisms underlying the observed changes. Upon rGDF11 treatment of the muscle-cell culture from old mice, the number of cellular mitochondria increased and multinucleate muscle fibers representing a syncytium of myoblasts began to form. It was supposed that rGDF11 not only induced mitochondrial response but also promoted the defective mitochondria removal from the muscle fiber of the old mice (Bitto and Kaeberlein, 2014; Sinha et al., 2014).

What are the mechanisms underlying the restoration of physiological activity of aging skeletal muscles and myocardium by the GDF11 protein?

It is common knowledge that skeletal muscles are a source of certain cytokines, the myokines, the synthesis rate of which decreases with age. Experiments with *Drosophila* showed that hyperexpression of Mnt, which is a transcription factor in the muscle tissue, increases longevity in flies. Mnt hyperexpression in muscles decreases the expression of the components of the nucleolus, the rRNA synthesis level, and nucleolus size in adipocytes. This process involves myoglianin, myostatin, and GDF11 (Fig. 3). Myoglianin protein produced by myocytes is able to bind to myostatin and GDF11 and affect the p38 transcription factor expression in adipocytes.

Overexpression of myoglianin in the muscles increases the life span and decreases the nucleolus size in adipocytes. Mitogen-activated proteinkinase MAPK activates p38 protein, while myoglianin shows the opposite effect in muscles. It is supposed that myoglianin plays a key role in integrating signaling processes in muscles and other tissues in aging (Demontis et al., 2014).

The process of the muscle mass regulation involves the myostatin and activin receptors, ActRII. These receptors mediate the GDF11 and activin effects on the skeletal muscles (Lach-Trifilieff et al., 2014). In their work, Lach-Trifilieff et al. (2014) blocked ActRII with bimagrumab and BYM338 antibodies and in this way prevented signal transduction to the target organs. BYM338 induced primary human skeletal myoblasts differentiation and counteracted the differentiation arrest caused by myostatin or activin A. In addition, BYM338 prevented atrophy promoted by ActRII via the inhibition of Smad 2/3 phosphorylation and also prevented myosin heavy chain degradation. BYM338 caused a skeletal muscle mass increase in mice. The

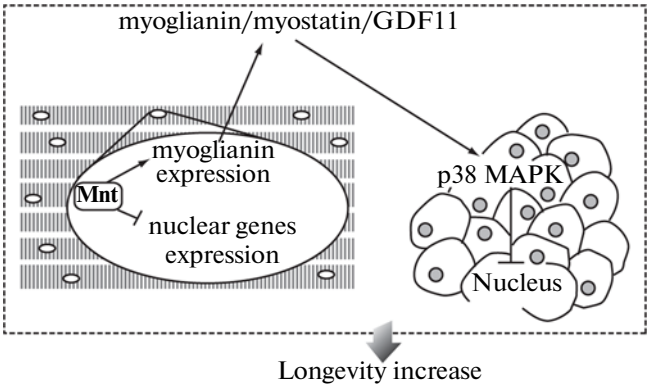

Fig. 3. Effects of the Mnt transcription factor and the GDF11 protein on the functional activity of the muscle (modified from Demontis et al., 2014). Adipocytes are depicted on the right; muscle fiber is on the left.

administration of antibodies against ActRII to mice in which myostatin was mutated led to muscle hypertrophy. BYM338 protected muscles from atrophy after the administration of glucocorticoids and prevented the development of muscular weakness. Thus, BYM338 may be used to treat diseases involving skeletal-muscle atrophy.

Follistatin, an inhibitor of TGF-β family proteins, is expressed on the myosphere-derived progenitor cells (MDPCs), which may give rise to cardiomyocytes (Nomura et al., 2008). At the same time, myostatin could mainly be found in the myogenic cells and mature skeletal muscles. Upon its action, the rate of the replicative growth of the MDPCs increased due to the inactivation of Smad 2/3 and cell cycle progression. Actin A or GDF11 inhibition induced MDPC proliferation via p21 inhibition and increasing the levels of Cdk 2/4 and cyclin D1.

The presented data indicate that follistatin increases the number of progenitor cells by neutralizing ActA and GDF11 and thus regulates MDPC growth in the skeletal muscle.

It is well known that the aging of multicellular organisms is accompanied by defects in repair pathways affecting hematopoiesis. At the same time, in the heteroparabiosis model, hematopoietic processes improved in elder mice, while the opposite effect was observed in the young animals (Villeda et al., 2011). In line 536, mice with myelodysplastic syndrome and anemia, impairments of the final stages of hematopoiesis are associated with the inhibition of the Smad 2/3 signaling and low GDF11 levels. In such mice, anemia could not be corrected by erythropoietin injections, while injection of the ACE 536 protein (trapping fusion) allowed complete healing of the anemia via restoration of the Smad 2/3 signaling pathway for GDF11 (Suragani et al., 2014).

It has been proved that GDF11 cytokine blocks erythrocyte maturation at the erythroblast stage and

causes early apoptosis in patients with β-thalassaemia. This occurs through the autocrine amplification loop, which produces oxidative stress and leads to α-globine precipitation. GDF11 expression in the spleen erythroblasts of the mice with thalassaemia and in the blood serum in patients with β-thalassaemia increased. GDF11 inactivation reduced oxidative stress and decreased membrane α-globin precipitation, which led to the enhancement of erythropoiesis (Dussiot et al., 2014).

Neuroprotective Properties of the GDF11 Protein

It was demonstrated that old mice improved their capabilities of learning and memorizing information after the three weeks of "parabiotic therapy." Injections of blood obtained from three-month-old mice rejuvenated elder mice's brains at the neuronal level, increasing their functional activity and simultaneously increasing the biochemical youth markers content. In the elder mice, long-term memory was improved significantly with the coincident activation of cognitive activity (animals more readily found the platform in the water labyrinth) and suppression of the alarm sense. The structural changes and activation of cognitive processes caused by the young blood and plasma were partly associated with the cAMP and cAMP response element-binding protein (CREB) activation in the hippocampus. This means that the administration of young blood and plasma leads to an increase in the synaptic plasticity and cognitive activity in elder mice. Therefore, the neurodegerative processes in the brain of elder mice appears to be reversible and may be compensated by GDF11 (Andersen and Lim, 2014).

Parabiosis between young and old animals causes changes in different structures of the nervous system of old mice. For example, neurons more readily form dendritic spines, the intensive growth of which accompanies learning in the animals, and new synapses (which are responsible for long-term memory) form in the hippocampus (Villeda et al., 2014). Factors promoting vasculature development in the brains of young mice induce in elder mice the differentiation of neurons and contribute to improved olfaction. The administration of GDF11 to old mice, like the transfusion of young mice blood, facilitates vasculature growth in the central nervous system, together with the development of neurons. It may be concluded that the suppression of age-related neurogenic degeneration by GDF11 lays the grounds for the development of new approaches to the therapy of neurodegenerative and vasculo-neuronal diseases in the elderly (Katsimpardi et al., 2014). It cannot be ruled out that Alzheimer's disease treatment in the future may be a combination of therapeutic effects of anti-amiloid peptide β antibodies and an increased expression of the gene encoding GDF11. It seems that such a therapeutic approach to Alzheimer's disease treatment may be quite justified by the GDF11 content, which is considerably decreased in the case of this disease (Sinha et al., 2014).

The study of mice embryos with the GDF11 (–/–) phenotype showed that the differentiation of the neurons in the spinal cord at the stage of precursors occurs more slowly in the absence of GDF11 than under normal conditions (Shi and Liu, 2011), while retardation of glial cell development was observed in the brain of GDF(–/–) mice. The proliferation of neurons in such mice is enhanced upon maximal GDF11 expression. Similar changes in neural precursors may be caused in vitro by the introduction of GDF11 to the corresponding cell culture. The GDF11 properties observed in the case of neuronal precursor cells are associated with its ability to induce p57 (Kip2) and p27 (Kip1) expression (Shi and Liu, 2011).

Scientists currently seek explanations for the mechanisms through which young blood factors, including GDF11, implement their geroprotective effects in elder mice. From the literature data presented, it may be concluded that the GDF11 protein possesses the properties of a neuroprotector and activates the functions of the cardiac and skeletal muscles. However, the considerable improvement of cognitive functions caused by GDF11 appears to be associated with synaptic plasticity restoration rather than with the enhanced development of neural cells.

Within several hours after its introduction, GDF11 is able to change the expression of 4700 genes. In particular, GDF11 regulates the expression of the cell cycle genes. Moreover, GDF11 inhibits the expression of genes associated with the regulation of cytoskeleton, including fascin and LIM, as well as the SH3 region protein 1 (LASP1) (Williams et al., 2013).

GDF11 regulates the genesis of olfactory receptor neurons, inhibiting the proliferation of the immediate neuronal precursors (INPs) that give rise to these cells (Gokoffski et al., 2011; Sinha et al., 2014; Wu et al., 2003). The signals from the neuronal precursors inhibit the generation of new neurons in the olfactory epithelium. GDF11 and its receptor molecules are expressed in large numbers in the olfactory cells. In in vitro experiments, GDF11 suppressed olfactory cell neurogenesis by inhibiting p27 (Kip1). In mice with GDF11 deficiency, there are considerably more precursor cells and neurons in the olfactory epithelium, while mice deficient in follistatin, the antagonist of GDF11, show retardation in the development of neuronal cells (Wu et al., 2003). GDF11 was proposed to be the regulator of the cell composition of the retinal ganglia (Kim et al., 2005). In the parabiosis model, elder mice not only demonstrate the development of the brain vasculature but also activation of the neuronal precursor cells and enhanced development of the olfactory cells, which results in improved olfaction (Katsimpardi et al., 2014).

It is well known that Foxg1 (the Winged Helix family transcription factor) promotes the development of t precursor neural structures. In mice with low Foxg1

level, development of the cerebral hemispheres and olfactory epithelium is impaired. It was supposed that such effects might be associated with increased GDF11 expression, which acts according to the feedback principle on the development of neurogenic structures and olfactory epithelium. However, defects in the cerebral hemispheres and olfactory epithelium development in the Foxg1 (–/–) mice are not associated with increased GDF11 expression. This means that GDF11 can not be the main factor causing the impairment of the nervous system and olfactory epithelium functioning observed in the Foxg1-deficient mice (Kawauchi et al., 2009).

In mice deficient for follistatin, Fst (–/–), the impairments of neuronal cell development could not be fully accounted for by the enhanced GDF11 activity (Gokoffski et al., 2011). GDF11, ActA, and Fst are the chief factors determining the development of neuronal and glial cells, including the receptor apparatus of the olfactory epithelium.

Effects of the GDF11 protein on pluripotent cells activation

The studies conducted by a group of scientists under the supervision of the Harvard Professor Ami Wagers (Sinha et al., 2014) showed that the GDF11 protein is able to induce functional activity of pluripotent cells in the aging organism. Upon blood transfusion from young mice to elder mice, pluripotent cells differentiated as myoid cells, activating the functions of not only the muscles, including myocardium, but of all other organs. This was accompanied by an increase in the synthesis of the GDF11 protein, the protein of youth.

It was determined that, in women with dysmenorrhea, an increase in the levels of the proinflammatory cytokines (IL1β, TNFα, IL6, and IL8) was shown to be accompanied by a decrease in the levels of the TGF-β family factors, including GDF11. During the secretory phase of menstrual cycle, an increase in the proinflammatory cytokine levels and a decrease in the growth factor levels were also observed; however, those shifts were less profound. The aforementioned factors play an important role in the processes of decidual membrane reorganization, as well as in the reparatory processes, and indirectly enhance primary dysmenorrhea.

The analysis of the presented data makes it possible to conclude that the various geroprotective effects of the GDF11 protein are associated with its affect on the genome by extending its functions onto pluripotent cells. Thus, a question arises as to whether epigenetic regulation of the GDF11 content in the blood of animals and human is possible. As a response to this question, Laviano (2014) notes that GDF11 in patients with the Alzheimer's disease may induce neuronal differentiation and modulate cellular synaptic plasticity. In patients with cachexia and muscular tissue atrophy associated with cancer, GDF11 is able to restore the functional activity of muscular tissue and the muscular tissue mass.

Promising directions of studying the geroprotective properties of the GDF11 protein

All of the presented data notwithstanding, the mechanisms of the GDF11 geroprotective action are rather poorly understood (Demontis et al., 2014). GDF11 is assumed to be a regulator of tissue aging that has been established as such in the course of evolution. The geroprotective effects caused by GDF11 may be associated with the p38 and MAPK nuclear protein cascade, which activates myostatin in mammals and myoglianin in *Drosophila*. Therefore, GDF11 and myostanin are able not only to prevent cardiac hypertrophy but also the aging of other tissues via activation of the p38–MAPK cascade and the regulation of the nucleolus functions. However, in mice, myostatin occurs mainly in the skeletal muscles, while GDF11 is widely expressed in different tissues. Although the highest level of the GDF11 expression is observed in spleen, the skeletal muscle is the most abundant tissue type in the organism, constituting 40% to 50% of the whole body mass. Thus, one future direction in the study of GDF11 may be concerned with answering the question of whether GDF11 expression in muscles can be regulated by the Mnt transcription factor, as is the case for myoglianin in *Drosophila*.

In addition, the search for the epigenetic mechanisms regulating the expression of the gene encoding the GDF11 protein also appears to be of importance. It is well known that the Lys-Glu and Ala-Glu-Asp-Gly peptides synthesized on the basis of the data obtained from studying the amino acid composition of the polypeptide complexes of the spleen and pineal gland are the epigenetic regulators of the expression of proinflammatory cytokines, as well as of the CCL11 chemokine, the protein marker of cell senescence (Khavinson et al., 2014). These short peptides increase life span and improve the life quality of animals and the telomere length in cell culture (Anisimov and Khavinson, 2010; Khavinson et al., 2013). For the Lys-Glu and Ala-Glu-Asp-Gly peptides, recognition sites were found in the promoter zones of various signal molecules (GCAG and ATTTC/G, respectively).

Pinealon, a short peptide enhancing physical performance and mental capacity and, thus, having effects similar to those of GDF11, has a Glu-Asp-Arg structure (Khavinson and Grigoriev, 2008). In the course of a clinical study, 72 individuals with craniocerebral injuries and cerebroasthenia, aged from 30 to 74 years, were treated orally with two capsules containing pinealon twice a day each day for 20–30 days (each capsule contained 0.1 mg of the drug substance) in addition to their standard treatment. The control group contained 37 individuals with the same pathologies who received the commonly accepted treatment.

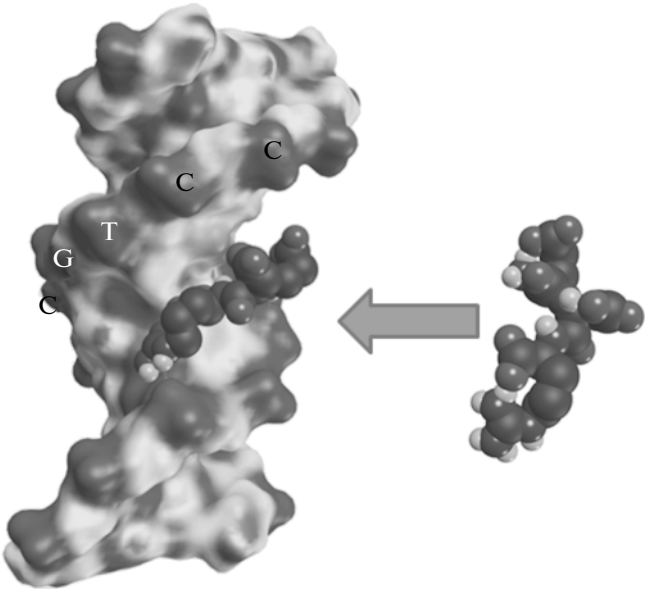

Fig. 4. Three-dimensional model of the DNA–peptide complex and the Glu-Asp-Arg peptide binding site (Khavinson et al., 2014). Left: DNA molecule with positively charged regions indicated by the light-gray and the negatively charged regions shown with the dark-gray. Right: the peptide molecule with the polar hydrogen atoms is shown in the light-gray and carbon and nitrogen atoms is shown in the dark-gray.

After treatment with pinealon, the patients noted improved memory, a decrease in the duration and intensity of head aches, and a more balanced emotional state. In patients with cranio-cerebral injuries treated orally with the tripeptide, the focal symptoms became less pronounced, while the verbal function in the case of motor and sensorial aphasia was improved. In patients with cerebroasthenia, oral treatment with pinealon resulted in fewer mistakes in the Bourdon test and an increased integral performance index (Morozov et al., 2011). Daily oral intake of pinealon (two capsules a day for two weeks) contributed to improvement of the antioxidant system functions in athletes, as well as to an enhanced adaptation to physical work, enhanced training rate, and increased metabolism rates. The higher energy supply of muscle tissue upon treatment with pinealon correlated with increased expression levels of *PPARA* and PPARG genes, which encode proteins that play a role in increasing the oxidative ability of skeletal muscles. At the same time, regulation of the organism's adaptability by the peptides was associated with an increase in the expression of the HSPA1A heat shock protein gene. It should be mentioned that pinealon treatment was associated in the athletes with the decreased rates of acute respiratory diseases, which was confirmed by examining their immune status. Athletes also showed an increased expression of immune-cell activation markers, including CD71, CD25, and HLA-DR, and a normal content of the M, G, and E class immunoglobulins (Khavinson et al., 2012). Seventy-five elderly patients were medicated orally with pinealon (two capsules per day every day for two weeks) to correct the psycoemotional state and the functional state of the central nervous system (Balashova et al., 2008). Upon the course of treatment, improvement of the short- and long-term memory and decreased severity indices were observed in patients.

The model of experimental prenatal hyperhomocysteinemia in rats was utilized to study the effects of the Glu-Asp-Arg peptide on the functional activity of the central nervous system. It is well known that in vivo induction of the oxidative stress is associated with an increase in the homocystein levels in blood, decreased cognitive abilities, and brain glutamatergic system impairment. The intramuscular injection of the Glu-Asp-Arg peptide in rats led to an improved spatial sense and improved learning in the progeny as judged by the Morris water-maze test results. It may be assumed that the protective action of the tripeptide consists of preventing the accumulation of reactive oxygen species in the neurons, thus increasing their stability, and blocking the interaction of homocystein and its derivatives with the glutamate receptors (Arutjunyan et al., 2012). A culture of cerebellum granular cells was used to assess how the Glu-Asp-Arg peptide affects the MAP kinase activation, the temporal profile of which determines the expression the genes of either adaptation or apoptosis. When the tripeptide was added to the cell culture, the lag period of MAP kinase activation appeared to last longer, which may be considered a protective effect against the toxic action of homocystein. Further, the tripeptide effects in the case of oxidative stress caused in neurons by uabain or hydrogen peroxide was studied. Pinealon caused a statistical decrease in the levels of reactive oxygen species in neurons (Khavinson et al., 2011).

It is possible to suppose that the neuroprotective peptide Glu-Asp-Arg may have binding sites in the *GDF11* gene promoter, as is the case with the aforementioned dipeptide and tripeptide (Fig. 4). The peptide binds DNA at the major groove side, forming a network of hydrogen bonds with the nitrogenous bases. The peptide presumably binds the CCTGC motif in the *GDF11* gene and thus participates in the regulation of this gene expression.

To test this hypothesis, the *GDF11* gene region corresponding to the promoter and extending from the –499 bp to the +100 bp relative to the transcription initiation site was analyzed. It appeared that there are two transcripts corresponding to the GDF11 protein, and accordingly, there are two promoter regions (Table). Presumable binding sites for the peptides under study were found in the promoters: namely, the TAAAG, GTTTA, CAAAT, and GAAAT motifs for the Ala-Glu-Asp-Gly peptide (Khavinson et al., 2012), the GACG and GCAG motifs for the Lys-Glu peptide (Khavinson et al., 2012), and the GCAGG, CGTCC,

Potential binding sites for the Lys-Glu, Glu-Asp-Arg, and Ala-Glu-Asp-Gly peptides in the promoter regions of the *GDF11* gene encoding the protein of youth and intellectual activity

GDF11 (NM_005811)	egulatory region of the gene from the −499 bp to the +100 pb (cDNA 5' ⟶ 3')
Transcript 1	TCTTCTTTTCCTCCTTTGTCTCTCCCTCTCTCCTCCTCCTTTTCCTATCTCTGTCT CTTCCCCCTTATCTCTCTGATTCTCCTTGGTCTCTCTGGCTCTGACTTTCCCTCT ATATCCGCCCCCCCGCCCCCTCATATCTCTGTCTCTTCATCTCTCTCTGGCCCTT GCTCCCTCATTCCCTCCCTCTCTCTATTCCTCGGCTCTCTCCGGCTCCCTCTCTC GCCTCGGATGACAGCGCTGCCTCTTTTGTTGGCTCC*GCAG*CCAATCGCGGCCG CT*GACG*ACACGGGGGCCGGGGCTA**TAAAG**GGCCTGGCCCGGGCTCGGGCCCC CCCAGCCGCCCGCCCCGGCCGCCCGCCCGCCCCGCCCGCGCGCCCGCCGCCC CCGGCCCCCCGGGTCCCCCCTCGGCCG*GGCAG*CCCCCAATCCCGCGCCGCCC GGACCCCCTCCTCCTCCCTCCCTCCTCCCTCCGCCCCCTCCCCGCGGGACTCC GG***CGTCC***CCGCCCCCCAGTCCTCCCTCCCCTCCCCTCCAGCATGGTGCTCGCG GCCCCGCTGCTGCTGGGCTT***CCTGC***TCCTCGCCCTGGAGCTGCGGCCCCGGGG GGAGGCGGCCG
Transcript 2	ACAGATGTATAGGAAGTGCTTGGATAGTTCCATTTTGCTGGGTGTTATTCCTAA TGGGAGTGAGGCAAGAAGCCTAGATCTGAATTCTG**GTTTA**CATAATGGGATAA ATTTAGCTCATGCTCCCTATCTGGACCCTGGGTTATCCCCTCTCTGAGGCCATC TGTGTTATTTTGTGGGGGAGGGATGTGCCAGGCTCTACCTGTCCA*GCAG*ATAA ATCAGA*GCAG*ATAGGGGAAAGGTGATGGAAGGGCAGCAGGTGTGATAAGAGG TATGGCTTCTA**TAAAG**AGCTTCAAAGATTCAGAAAATGTTGGAGCCTTATATGC TGGGAAAAGTTGGACAGTAAGGATGGTGGTGGATGAATAATTTT***GCAGG***TTAT CTGGTAGACAGGAACCTATATATTAGGG**CAAAT**GGATTAGGAAATGGACACAG ATCAGTAAGGCCTTATAGGGCCATCATCCTAAAGAGGAAGTGCTGTTTTAGGT ACCGGAGACATGGTATAAGATAGGCATGGGAGAAGGG**TAAAG**AAGAACTGGA AAATCAGGCTGAGAAGTCCAAAATTGCCAGTGCCACCCAGGACTACTGATCC CCTACACAAACACCCTT

Binding sites are shown in bold for the Ala-Glu-Asp-Gly peptide, in italics with underlining for the Lys-Glu peptide, and in bold italics with underlining for the Glu-Asp-Arg peptide. The gene accession number in the GenBank database is given in parenthesis. The longest region contains two sites.

and CCTGC motifs for pinealon (Khavinson et al., 2014).

CONCLUSIONS

It was determined that the promoter regions of the *GDF11* gene contain all of the possible binding sites for the Lys-Glu, Glu-Asp-Arg, and Ala-Glu-Asp-Gly peptides, which presumably epigenetically affect the expression of this gene without disturbing the DNA structure.

The data provided in the current review indicate that the GDF11 protein identified in the peripheral blood of animals and human has geroprotective properties. GDF11 contributes to the increase in myocardium and skeletal muscle functional activity in aging by regulating the MAPK–p38–myoglianin cascade. Moreover, GDF11 shows marked neuroprotective properties, changing the activity of the p57 (Kip2) and p27 (Kip1) transcription factors, which implies its ability to activate the differentiation of neurons and increase their neuroplasticity. Since the most frequent age-related pathologies are cardiovascular and neurodegenerative diseases, GDF11 may be considered a potential target for geroprotective therapeutic compounds or as an independent biologically active compound. The short Glu-Asp-Arg peptide may be considered an example of a compound that could potentially stimulate the synthesis of GDF11; its neuroprotective effects on the organism as a whole and its signal molecules are quite similar to those of GDF11.

It may be proposed, therefore, that the Lys-Glu, Glu-Asp-Arg, and Ala-Glu-Asp-Gly peptides could be potential regulators of the GDF11 level in blood.

REFERENCES

Andersen, R.E. and Lim, D.A., An ingredient for the elixir of youth, *Cell Res.*, 2014, vols. 1–2, pp. 1345–1356.

Anisimov, V.N. and Khavinson, V.K., Peptide bioregulation of aging: results and prospects, *Biogerontology*, 2010, vol. 11, pp. 139–149.

Arutjunyan A., Kozina L., Stvolinskiy S., et al., Pinealon protects the rat offspring from prenatal hyperhomocysteinemia, *Int. J. Clin. Exp. Med.*, 2012, vol. 5, no. 2, pp. 179–185.

Balashova, S.N., Zhernakov, G.L., and Dudkov, A.V., Implementation of peptide bioregulators for elderly people with psychoemotional disorders, *Usp. Gerontol.*, 2008, vol. 21, no. 3, pp. 448–452.

Bitto, A. and Kaeberlein, M., Rejuvenation: it's in our blood, *Cell Metab.,* 2014, vol. 20, no. 1, pp. 2–4.

Brack, A.S., Ageing of the heart reversed by youthful systemic factors! *EMBO J.*, 2013, vol. 32, no. 16, pp. 2189–2190.

Demontis, F., Patel, V.K., Swindell, W.R., and Perrimon, N., Intertissue control of the nucleolus via a myokine-dependent longevity pathway, *Cell Rep.*, 2014, vol. 7, no. 5, pp. 1481–1494.

Donskov, S.I. and Yagodinskii, V.N., Last days of A.A. Bogdanov: chronicle of tragedy, *Vestn. Sluzhby Krovi Ross.*, 2006, vol. 1, pp. 1–8.

Dussiot, M., Maciel, T.T., Fricot, A., et al., An activin receptor IIA ligand trap corrects ineffective erythropoiesis in β-thalassemia, *Nat. Med.*, 2014, vol. 20, no. 4, pp. 398–407.

Gokoffski, K.K., Wu, H.H., Beites, C.L., et al., Activin and GDF11 collaborate in feedback control of neuroepithelial stem cell proliferation and fate, *Development*, 2011, vol. 138, no. 19, pp. 4131–4142.

Katsimpardi, L., Litterman, N.K., Schein, P.A., et al., Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors, *Science*, 2014, vol. 344, pp. 630–634.

Kawauchi, S., Kim, J., Santos, R., et al., Foxg1 promotes olfactory neurogenesis by antagonizing Gdf11, *Development*, 2009, vol. 136, no. 9, pp. 1453–1464.

Khavinson, V.Kh., Grigoriev, E.I., Malinin, V.V., and Ryzhak, G.A., EU Patent ÅÀ 010157, 2008.

Khavinson, V.Kh., Kuznik, B.I., Tarnovskaya, S.I., and Linkova, N.S., Peptides and CCL11 and *HMGB*1 as molecular markers of aging: literature review and own data, *Adv. Gerontol.*, 2015, vol. 5, no. 3, pp. 133–140.

Khavinson, V.Kh., Lin'kova, N.S., Tarnovskaya, S.I., Umnov, R.S., Elashkina, E.V., and Durnova, A.O., Short peptides stimulate serotonin expression in cells of brain cortex, *Bull. Exp. Biol. Med.*, 2014, vol. 157, no. 1, pp. 77–80.

Khavinson, V., Ribakova, Y., Kulebiakin, K., et al., Pinealon increases cell viability by supression of free radical levels and activating proliferative processes, *Rejuven Res.*, 2011, vol. 14, no. 5, pp. 535–541.

Khavinson, V.K., Tarnovskaya, S.I., Linkova, N.S., et al., Short cell-penetrating peptides: a model of interactions with gene promoter sites, *Bull. Exp. Biol. Med.*, 2013, vol. 154, no. 3, pp. 403–410.

Khavinson, V.Kh., Trofimova, S.V., and Viner, I.A., *Metodika povysheniya rezervnykh vozmozhnostei organizma sportsmenov vysokoi kvalifikatsii, spetsializiruyushchikhsya v slozhnokoordinatsionnykh vidakh sporta, s pomoshch'yu peptidnykh bioregulyatorov. Metodicheskie rekomendatsii* (A Method for Improvement of Reserves of Organisms of Sportsmen Specializing in Complex Sport Types Using Peptide Bioregulators: Methodological Recommendations), St. Petersburg: Inst. Bioregul. Gerontol., 2012.

Kim, J., Wu, H.-H., Lander, A.D., et al., GDF11 controls the timing of progenitor cell competence in developing retina, *Science,* 2005, vol. 308, pp. 1927–1930.

Kolchinskaya, A.Z., *Kislorod. Fiziologicheskoe sostoyanie. Rabotosposobnost'* (Oxygen. Physiological State. Workability), Kyiv: Naukova Dumka, 1991.

Lach-Trifilieff, E., Minetti, G.C., Sheppard, K., et al., An antibody blocking activin type II receptors induces strong skeletal muscle hypertrophy and protects from atrophy, *Mol. Cell. Biol.,* 2014, vol. 34, no. 4, pp. 606–618.

Laviano, A., Young blood, *N. Engl. J. Med.*, 2014, vol. 371, pp. 573–575.

Loffredo, F.S., Steinhauser, M.L., Jay, S.M., et al., Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy, *Cell*, 2013, vol. 153, no. 4, pp. 828–839.

Morozov, V.G., Ryzhak, G.A., Malinin, V.V., and Rutkovskaya, V.N., *Tsitogeny. Biologicheski aktivnye dobavki k pishche. Metodicheskie rekomendatsii* (Cytogenes. Biological Active Supplements to Food: Methodological Recommendations), St. Petersburg: Kosta, 2011.

Nomura, T., Ueyama, T., Ashihara, E., et al., Skeletal muscle-derived progenitors capable of differentiating into cardiomyocytes proliferate through myostatin-independent TGF-beta family signaling, *Biochem. Biophys. Res. Comm.*, 2008, vol. 365, no. 4, pp. 863–869.

Pottgiesser, T., Sottas, P.-E., Echteler, T., et al., Detection of autologous blood doping with adaptively evaluated biomarkers of doping: a longitudinal blinded study, *Transfusion*, 2011, vol. 51, no. 8, pp. 1707–1715.

Shi, Y. and Liu, J.-P., Gdf11 facilitates temporal progression of neurogenesis in the developing spinal cord, *J. Neurosci.*, 2011, vol. 31, no. 3, pp. 883–893.

Sinha, M., Jang, Y.C., Oh, J., et al., Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle, *Science*, 2014, vol. 344, pp. 649–652.

Suragani, R., Cadena, S.M., Cawley, S.M., et al., Transforming growth factor-@ superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis, *Nat. Med.*, 2014, vol. 20, no. 4, pp. 408–414.

Villeda, S.A., Luo, J., Mosher, K.I., et al., The ageing systemic milieu negatively regulates neurogenesis and cognitive function, *Nature,* 2011, vol. 477, pp. 90–94.

Villeda, S.A., Plambeck, K.E., Middeldorp, J., et al., Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice, *Nat. Med.*, 2014, vol. 20, no. 6, pp. 659–663.

Williams, G., Zentar, M.P., Gajendra, S., et al., Transcriptional basis for the inhibition of neural stem cell proliferation and migration by the TGF-β-family member GDF11, *PloS One,* 2013, vol. 8, no. 11, p. e78478.

Wu, H.H., Ivkovic, S., Murray, R.C., et al., Autoregulation of neurogenesis by GDF11, *Neuron*, 2003, vol. 37, no. 2, pp. 197–207.

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SPELL: 1. intraperitoneal