Mol Neurobiol
DOI 10.1007/s12035-016-0017-x

Tandem Delivery of Multiple Therapeutic Genes Using Umbilical Cord Blood Cells Improves Symptomatic Outcomes in ALS

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Received: 11 June 2016 / Accepted: 28 July 2016

Abstract Current treatment options of chronic, progressive degenerative neuropsychiatric conditions offer only marginal efficacy, and there is no therapy which arrests or even reverses these diseases. Interest in genetic engineering and cell-based approaches have constantly been increasing, although most of them so far proved to be fruitless or at best provided very slight clinical benefit. In the light of the highly complex patho-mechanisms of these maladies, the failure of drugs aimed at targeting single molecules is not surprising. In order to improve their effectiveness, the role of a unique triple-combination gene therapy was investigated in this study. Intravenous injection of human umbilical cord blood mononuclear cell (hUCBMC) cotransduced with adenoviral vectors expressing vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF), and neural cell adhesion molecule (NCAM) resulted in prominent increase of life span and performance in behavioral tests in amyotrophic lateral sclerosis (ALS). Expression of the recombinant genes in hUCBMCs was confirmed as soon as 5 days after transduction by RT-PCR, and cells were detectable for as long as 1 month after grafting in lumbar spinal cord by immunofluorescent staining. Xenotransplantation of cells into mice blood without any immunosuppression demonstrated a high level of hUCBMCs homing and survivability in the central nervous system (CNS), most conspicuously in the spinal cord, but not in the spleen or liver. This study confirms an increased addressed homing and notable survivability of triple-transfected cells in lumbar spinal cord, yielding a remarkably enhanced therapeutic potential of hUCBMCs overexpressing neurotrophic factors.

Keywords Cell-mediated gene therapy · Adenoviral vector · Amyotrophic lateral sclerosis (ALS) · Human umbilical cord blood mononuclear cell (hUCBMC) · Vascular endothelial growth factor (VEGF) · Glial cell-derived neurotrophic factor (GDNF) · Neural cell adhesion molecule (NCAM)

Introduction

At present, there is no efficient approach to slow down or even stop the death of brain cells that is a characteristic feature of most degenerative neuropsychiatric disorders. Gene- and cell-based therapies are gaining much interest but with little clinical benefit. The use of modified cells expressing genes of several growth factors with neurotrophic functions such as insulin-like growth factor-1 (IGF_1), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), fibroblast growth factor-2 (FGF_2), angiogenin (ANG), and many others have showed some effect [1]. Viral vectors carrying these therapeutic genes may be directly injected intrathecally, intravenously, or intramuscularly (direct gene therapy), but these may be transferred by stem cells or specific mature cells or by mixed population thereof (cell-mediated gene therapy).

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Published online: 06 August 2016

The advantages of direct or cell-mediated gene therapy are obscure, although investigators demonstrate some efficacy of both types of gene therapy. Cell-mediated gene delivery is based on ex vivo transduction of auto- or allogenic cells. Stem cells (neural, mesenchymal) [2, 3] or different somatic cells (fibroblasts, mononuclear cells from umbilical cord blood) [4, 5] may be used as bioreactors. Foundation of numerous banks for collection of human umbilical cord blood mononuclear cells (hUCBMC) has given rise to intensive study of hUCBMCs in cell therapy of degenerative disorders [6, 7]. Previously, we have shown that hUCBMCs genetically engineered for tandem delivery of neurotrophic factor (VEGF or GDNF) in combination with neuronal cell adhesion molecule (NCAM) improve symptomatic outcomes and increase life span in ALS mouse model [8]. In the present research, we developed innovative gene-cell constructs for ALS treatment based on hUCBMC genetically engineered to simultaneous overexpress human VEGF, GDNF, and NCAM genes. The efficacy of hUCBMC-mediated gene therapy was examined using behavioral tests, life span measurement, and immunofluorescent analysis of the hUCBMC homing, survival, and therapeutic gene expression in ALS.

Material and Methods

Gene Modification of hUCBMC

Recombinant adenoviruses Ad5-EGFP, Ad5-GDNF, Ad5-NCAM1, and Ad5-VEGF165 were generated as described previously [8]. Gene modification of human umbilical cord blood cells (hUCBMC) was performed on the basis of stem cell bank of Kazan State Medical University. After purification, fraction of mononuclear cells from human umbilical cord blood was seeded on 10-cm culture dish and transduced with recombinant adenoviruses with MOI 10. Before trasplantation the cells were incubated for 12−16 h in RPMI-1640 medium (Sigma, USA) supplemented with 10 % FBS and a mixture of antibiotic penicillin and streptomycin (100 U/ml and 100 μg/ml, respectively).

Molecular Analysis of Recombinant Gene Expression

hUCBMC simultaneously transduced with Ad5-VEGF165, Ad5-GDNF, and Ad5-NCAM1 were collected 5 days after culturing. RNA isolation was performed with RNA isolation kit from cell cultures according to the producer's instructions (Sileks, Russia) and followed by synthesis of cDNA with use of 100 U Maxima Reverse Transcriptase (Thermo Scientific, USA). Samples of cDNA were analyzed by CFX 96 Real-Time PCR System (BioRad, USA). Nucleotide sequences of primers are shown in Table 1. The level of mRNA was normalized using 18S rRNA as housekeeping gene. Standard curves for relative quantitation of the NCAM1, VEGF, and GDNF were generated using serial dilutions of plasmid DNA with corresponding cDNA inserts. Expression level of target genes in non-transduced hUCBMC was considered as 100 %. All RT-PCR reactions were performed in triplicates. hUCBMC genetically engineered to express GFP were examined using fluorescent microscope Axio Observer Z1 (Carl Zeiss, Germany).

Animals and Treatments

Transgenic for mutant human SOD1 gene (B6SJL-TG(SOD1-G93 A)dl1Gur/J) mice were obtained from Jackson

Table 1 Nucleotide sequences of the primers and probes for real-time PCR

Name of the primer	Nucleotide sequence
VEGF-TM-Forward	ATCACCATGCAGATTATGCG
VEGF-TM-Reverse	TGCATTCACATTTGTTGTGC
VEGF-TM-Probe	[FAM]TCAAACCTCACCAAGGCCAGCA[BH1]
GDNF-TM-Forward	CGCTGAGCAGTGACTCAAAT
GDNF-TM-Reverse	CGATTCCGCTCTCTTCTAGG
GDNF-TM-Probe	[FAM]TCCATGACATCATCGAACTGATCAGG[BH1]
18S-TM-Forward	GCCGCTAGAGGTGAAATTCTTG
18S-TM-Reverse	CATTCTTGGCAAATGCTTTCG
18S-TM-Probe	[HEX]ACCGCGCAAGACGGACCAG[BH2]
NCAM1-TM-Forward	AGATGAGGGCACTTATCGCT
NCAM1-TM-Reverse	GATGGTAGGTGGCACATTCA
NCAM1-TM-Probe	[FAM]CCGTGCCAGGATTCTGCCCT[BH1]
EGFP-TM-Forward	AGCAAAGACCCCAACGAGAA
EGFP-TM-Reverse	GGCGGCGGTCACGAA
EGFP-TM-Probe	[FAM]CGCGATCACATGGTCCTGCTGG[BH1]

Laboratory (Bar Harbor, USA). Expression of mutant form of human copper/zinc superoxide dismutase-1 (hSOD1) develops in mice symptoms similar to amyotrophic lateral sclerosis (ALS) in humans. ALS mice were bred at animal facility of Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (Russia) and presymptomatic mice were delivered to Kazan State Medical University and housed one per cage under standard laboratory conditions, with a 12-h light/dark schedule and unlimited access to food and water. Mature 26-week-old male and female ALS mice were randomly assigned to three groups according to different treatments: (1) control group ($n = 8$)—injection of saline, (2) hUCBMC-GFP group ($n = 7$)—transplantation of genetically engendered hUCBMC transduced with Ad5-EGFP, (3) hUCBMC-VGN group ($n = 11$)—transplantation of genetically engendered hUCBMC transduced with Ad5-VEGF, Ad5-GDNF, and Ad5-NCAM. Presymptomatic 29-week-old ALS mice retroorbitally received 2×10^6 genetically engineered hUCBMC in 100 μl of saline. Animal treatment protocol was approved by the Kazan State Medical University Animal Care and Use Committee.

Behavioral Tests

Two weeks before hUCBMC transplantation, presymptomatic 27-week-old mice were trained to perform open field and paw grip strength tests as described previously [8]. Shortly, in the open field test, mice were placed to the arena for 3 min and the number of crossed lines (horizontal activity), vertical stands (vertical activity), and the number of explored holes in the arena floor (exploration activity) were analyzed. In the paw grip strength test, mice were allowed to grip a metal grid with all four paws, and the grid was slowly rotated over so that the mice were turned upside down. The time which mice were hanging to metal grid was evaluated and the best result from three attempts was recorded [9]. Behavioral tests were performed at 4- and 8-week time points after hUCBMC transplantation. Each of the tests was repeated twice per examination week. Following the behavioral testing, ALS mice become paralyzed and reached the end point of disease which was determined when the animals could no longer forage for food or water. The end point of each mouse was monitored as a life span.

Immunofluorescence

At the terminal stage of the disease, the mice under ketamine-xylazine anesthesia were transcardially perfused first with cold PBS and then with cold 4 % paraformaldehyde in PBS (pH 7.4). The whole spinal cord was removed, postfixed with paraformaldehyde overnight at 4 °C and then immersed in 30 % sucrose solution in PBS (pH 7.4) at 4 °C. Frozen free-floating (20 μm) serial cross sections of lumbar spinal cord were prepared. To demonstrate transplanted hUCBMC and to verify expression of therapeutic genes encoding VEGF, GDNF, and NCAM in gene-modified hUCBMC, the lumbar spinal cords of ALS mice were subjected to immunofluorescent staining. After blocking of non-specific binding sites with normal donkey serum, the primary antibodies (Ab) were applied overnight at 4 °C. Afterwards, sections were incubated with proper secondary Ab for 2 h at room temperature and cell nuclei were counterstained with bis-benzimide solution (Hoechst 33258 dye, Sigma-Aldrich, USA). Finally, sections were mounted on SuperFrost®Plus glass slides, embedded in anti-quenching medium and examined with a laser scanning microscope (LSM 510-Meta, Carl Zeiss, Germany). hUCBMC (HNA-positive cells) were counted in 100 sections from each mouse. The list of the used antibodies is presented in Table 2. In mice from hUCBMC-GFP and hUCBMC-VGN groups, the spleen and liver were additionally examined with

Table 2 Primary and secondary antibodies for immunofluorescence

Antigene	Host	Dilution	Company
[a]GDNF	Rabbit	1:100 1:150	Santa Cruz
[a]VEGF	Goat	1:125	Sigma
[a]NCAM1, PE conjugated	Mouse	1:100 1:150	Sorbent (Moscow, Russia)
[a]Human nuclei antigen (HNA)	Mouse	1:150	Millipore
[b]Anti-rabbit Alexa 488	Donkey	1:1000 1:150	Invitrogen
[b]Anti-mouse Alexa 647	Donkey	1:150	Invitrogen
[b]Anti-goat Alexa 488	Donkey	1:150	Invitrogen

[a] Primary antibodies

[b] Secondary antibodies

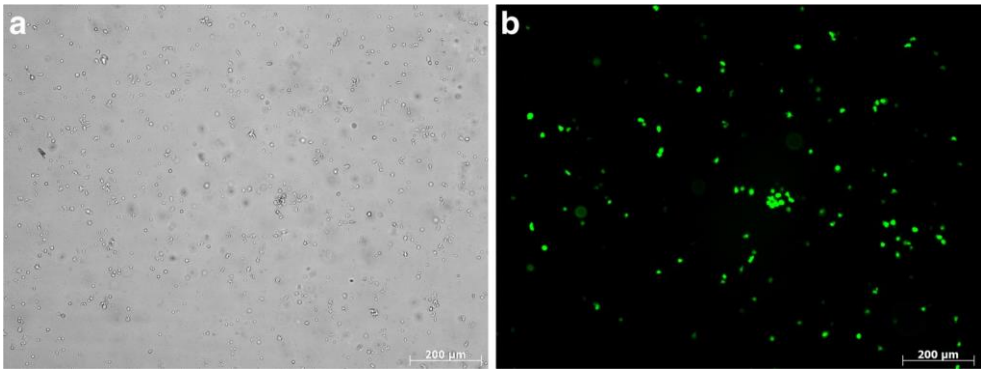

Fig. 1 Immunofluorescent study of the reporter GFP gene expression in hUCBMC 5 days after cell transduction with recombinant adenovirus Ad5-EGFP (MOI 10) in bright field channel (a) and green channel (b)

antibodies to human nuclei antigen (HNA) to demonstrate the homing and survivability of hUCBMC in parenchymal organs of the mice at the terminal stage of the disease in the first month of the experiment.

Statistical Analysis

All data are presented as mean ± S.D. with statistical significance assessed by Student's *t* test, with $P < 0.01$ being regarded as a statistically significant difference.

Results

In Vitro Study of hUCBMC

The effectiveness of hUCBMC transduction with Ad5-EGFP or hUCBMCs transduction simultaneously with Ad5-VEGF165, Ad5-GDNF and Ad5-NCAM1 were examined 5 days after cell culturing. hUCBMC transduced with Ad5-EGFP were studied with fluorescent microscopy. Cytoplasm of the transduced cells showed intensive green fluorescence (Fig. 1). In hUCBMC transduced with Ad5-VEGF165, Ad5-GDNF, and Ad5-NCAM1, the level of the therapeutic genes mRNA was evaluated by RT-PCR. Gene expression data normalized to 18S ribosomal RNA level demonstrated 91, 64, and 58 times higher level of mRNA for VEGF, GDNF, and NCAM correspondingly and relatively to non-transduced cells (Fig. 2). Herein, we demonstrate an evidence of the effectiveness of simultaneous transduction of hUCBMC with tree adenoviral vectors carrying *vegf165*, *gdnf*, and *ncam1* genes.

Behavioral Tests

The effectiveness of intravenous hUCBC transplantation on the disease development in ALS mice was evaluated with open field and paw grip strength behavioral tests. In presympomatic mice, 2 days before hUCBMC transplantation, mice from all three groups had the similar results without significant difference ($P > 0.05$). In mice from control group, open field test showed the following number of attempts: horizontal activity—70.9 ± 2.7, vertical activity—10.2 ± 2.1, and exploration activity—6.3 ± 1.4. The paw grip strength behavioral test was 56.6 ± 4.2 s. Four weeks after saline or hUCBMC transplantation, the drastic decrease in vertical activity in open field and in paw grip strength tests were observed in control and hUCBMC-GFP experimental groups relatively to the hUCBMC-VGN group. The horizontal and exploration activities of the ALS mice in the open field did not markedly differ at 4-week time point in all experimental groups in comparison with those of the presymptomatic mice (Fig. 3).

At 8-week time point in open field test, vertical, horizontal, and exploration activities have continued to decrease but the meanings were significantly better in the hUCBMC-VGN group in comparison with those in the control and hUCBMC-GFP groups. In the paw grip strength test, the prominent reduction of the experimental time in all three

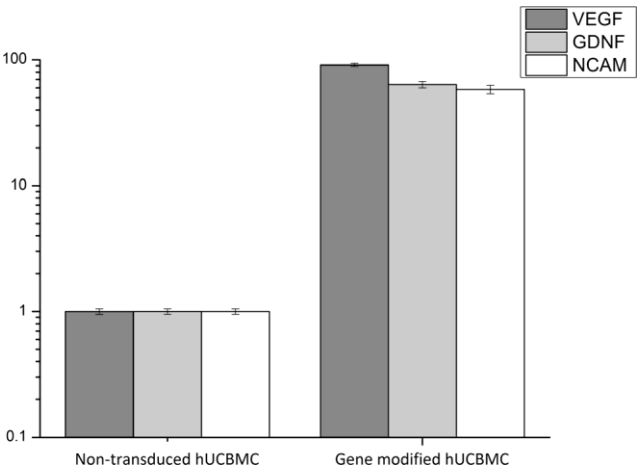

Fig. 2 RT-PCR analysis of therapeutic gene expression in hUCBMC 5 days after cell transduction. Data presented as mean ± S.D. for three independent experiments; the differences between transduced and non-transduced hUCBMC are statistically significant ($P < 0.01$)

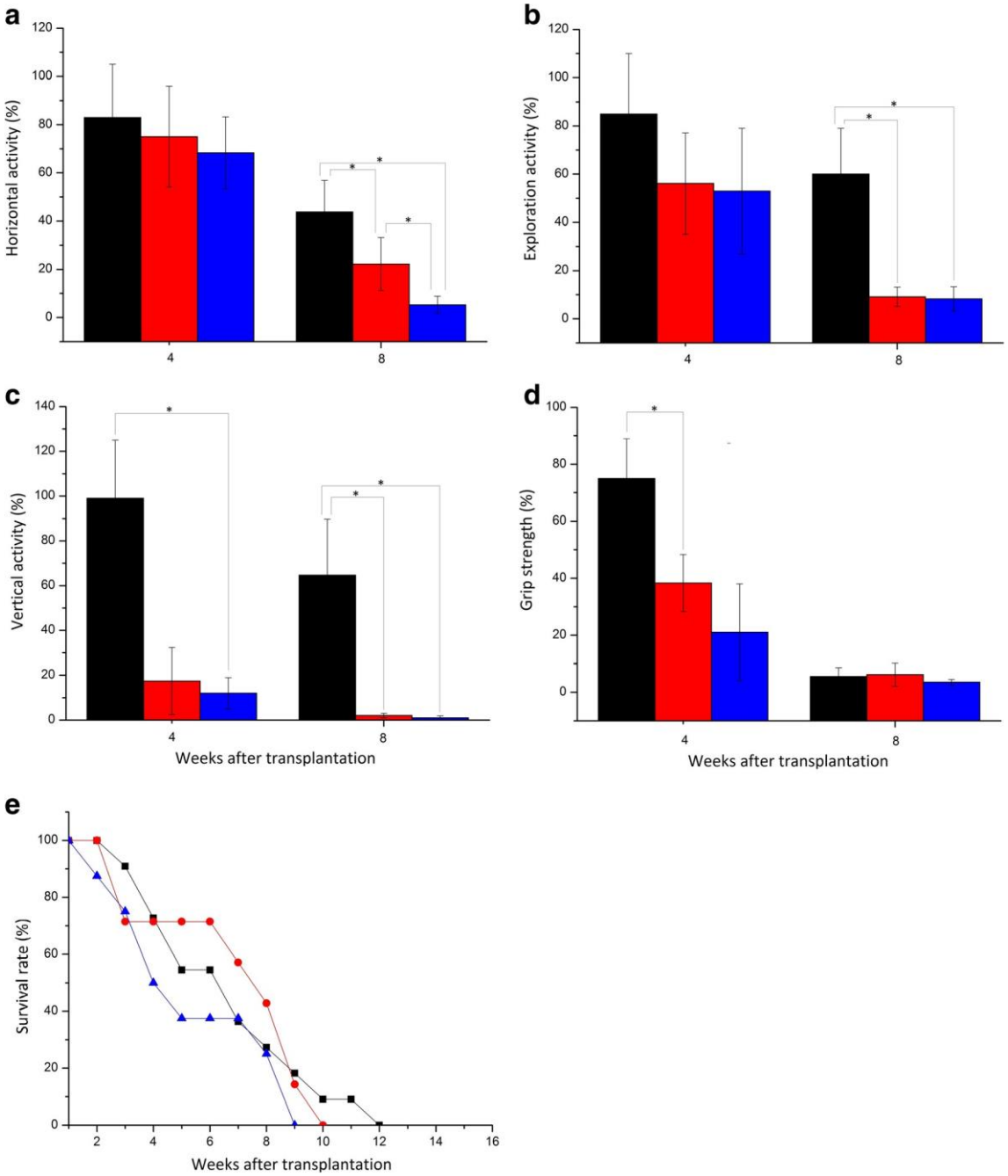

Fig. 3 Behavioral test performance and survival rates of ALS mice at 4 and 8 weeks after genetically modified hUCBMC transplantation. a Horizontal activity. b Exploration activity. c Vertical activity. d Grip strength test. Average meanings with standard deviation are shown; *asterisk* represents statistically significant differences between mice groups ($P < 0.05$). e Survival rates of ALS mice (percentage of alive mice to initial amount of animals in group) is shown. X-axis—weeks after hUCBMC transplantation, Y-axis—parameters as percentage of initial. Experimental groups: hUCBMC-VGN (*black*), hUCBMC-GFP (*red*), and control (*blue*)

groups was observed. In general, hUCBMC-VGN group of ALS mice had slower development of the disease symptoms.

Evaluation of the survival rates of ALS mice between three experimental groups (Fig. 3) showed two crucial time points: 4 and 8 weeks after hUCBMC transplantation. However, the survival of mice from hUCBMC-VGN group was higher compared to control and hUCBMC-GFP groups. It is worth to mention that hUCBMC-VGN mice lived for 4 weeks longer than the control mice and for 2 weeks longer than the hUCBMC-GFP mice.

Thus, analysis of the behavioral status of ALS mice demonstrated the slower rate of ALS symptom development and higher rate of survival of mice treated with hUCBMC overexpressing therapeutic genes VEGF, GDNF, and NCAM.

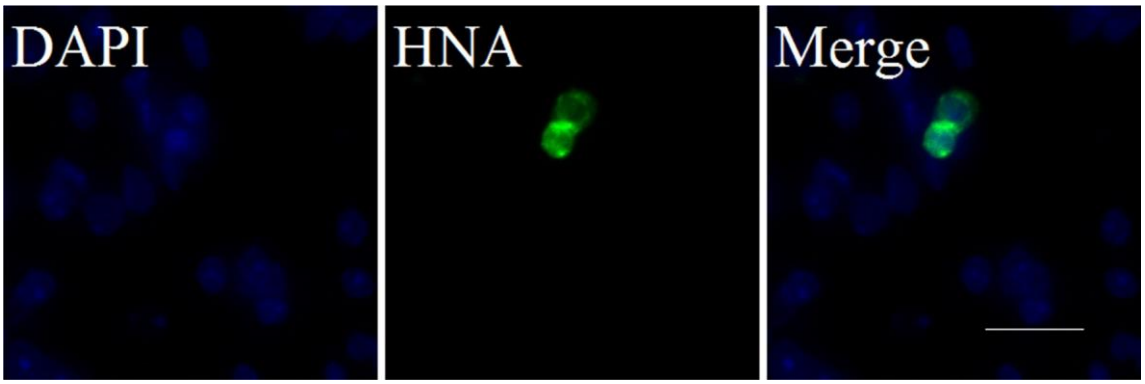

Fig. 4 Visualization of grafted cells in ALS mice lumbar spinal cord 1 month after hUCBMC transplantation. Immunofluorescent staining demonstrates HNA^+-cells (*green*). Nuclei are counterstained with DAPI (*blue*). *Scale bar* 20 μm

Immunofluorescent Study of Lumbar Spinal Cord

Immunofluorescent analysis of lumbar spinal cord was performed in mice sacrificed at the terminal stage of the disease between 3 and 9 weeks after hUCBMC transplantation. Using Abs to HNA, we have evaluated homing and survivability of hUCBMCs in spinal cord. HNA-positive cells were revealed in gray and white matter in both experimental groups (Fig. 4). The drastic difference in the number of HNA^+-cells were found in the lumbar spinal cord of the mice from hUCBMC-GFP and hUCBMC-VGN groups. In mice sacrificed at 7–8 weeks after hUCBMC transplantation, the number of HNA^+-cells were higher in hUCBMC-VGN group (Fig. 5). The higher level of survivability of the grafted cells in spinal cord of ALS mice from hUCBMC-VGN group demonstrates to the important role of recombinant NCAM expression in hUCBMC for their survival.

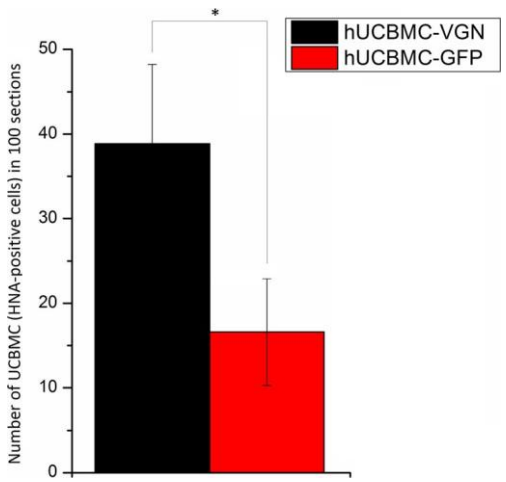

Fig. 5 The number of hUCBMC (HNA-positive cells) in lumbar spinal cord of ALS mice 7–8 weeks after cell transplantation. *Black column*—hUCBMC-VGN ($n = 3$), *red column*—hUCBMC-GFP ($n = 3$). HNA-positive cells were counted in 100 sections from each mouse ($^*P < 0.05$)

To demonstrate therapeutic gene expression in HNA^+-cells from hUCBMC-VGN group, the double immunofluorescent staining was performed. The lumbar spinal cord of ALS mice was stained in combination of Abs to HNA with VEGF, GDNF, or NCAM. Microscopic analysis revealed HNA^+/$VEGF^+$ cells, HNA^+/$GDNF^+$ cells, and HNA^+/$NCAM^+$ cells 3–4 weeks after hUCBMC transplantation (Fig. 6). Round-shaped view of the cells corresponds to the morphology of the cord blood mononuclear cells with approximately 8–10 μm in diameter. Big round nuclei are surrounded by a thin rim of cytoplasm and because of the spinal cord section with thickness (20 μm), we observe overlapped cytoplasmic and nuclear fluorescence.

Investigation of the spleen and liver from mice of hUCBMC-GFP and hUCBMC-VGN groups during the first month after hUCBMC transplantation HNA-positive cells in parenchymal organs were not observed (data not shown). Xenotransplantation hUCBMC into mice blood without immunosuppression demonstrated a high level of hUCBMC homing and survivability in CNS but not in the spleen or liver. These data demonstrate that hUCBMC may effectively cross the BBB and the immunoprivilege feature of the CNS provides their defense from the mice immune system.

Discussion

Cellular strategy to motor neuron disease therapy is mostly based on the use of stem cells (neural and mesenchymal) and induced pluripotent stem cells [10]. In the past decade, researchers made popular for the cell therapy in different neurological conditions application of the hUCBMC [11, 12]. To enhance the effectiveness of the cell therapy in neurodegenerative disorders, the approaches to combine cellular and gene therapies were proposed. For the first time, we demonstrated hUCBMC-based delivery of three therapeutic genes into the site of spinal motor neuron degeneration. Neural cell adhesion

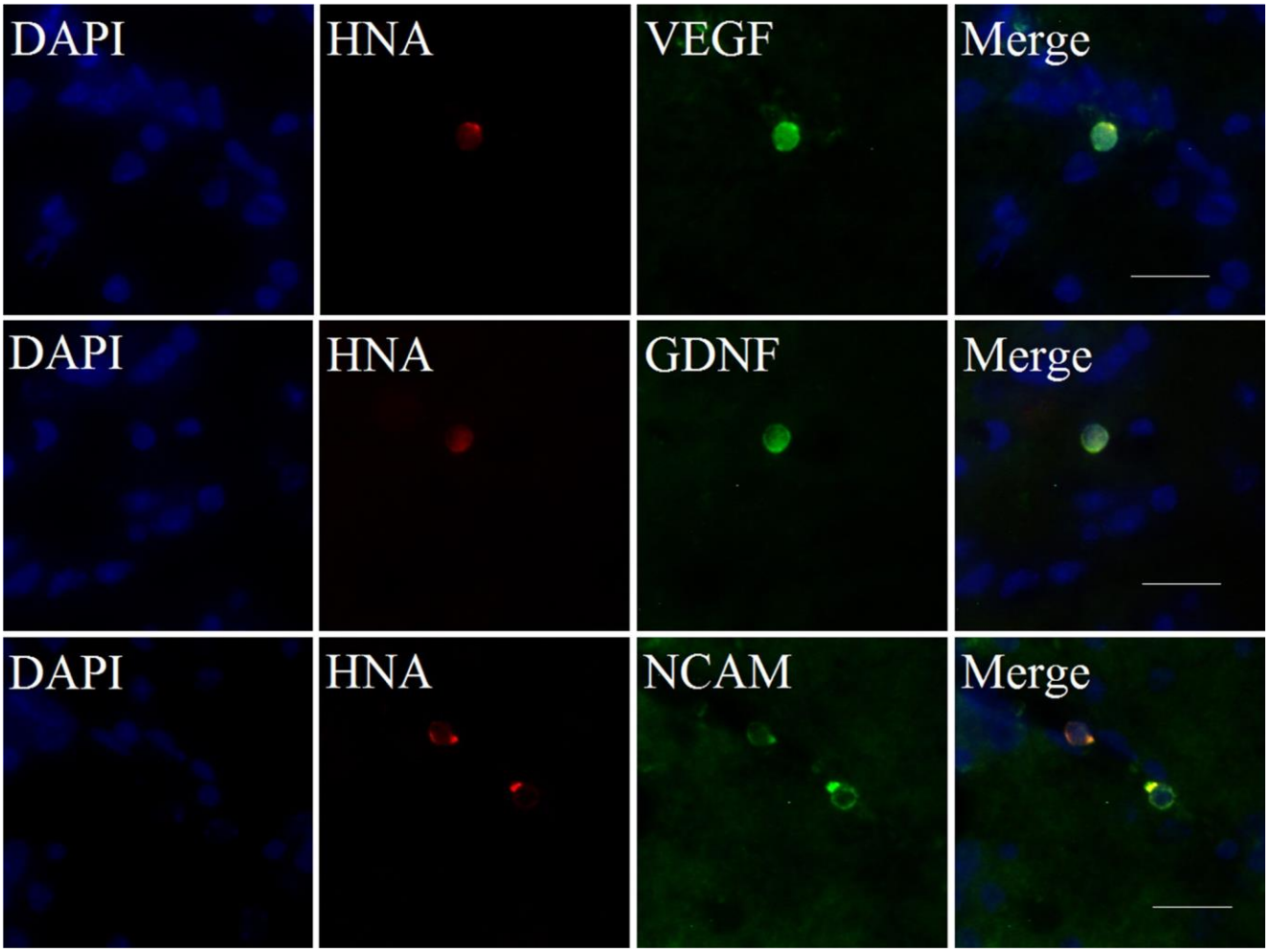

Fig. 6 Evidence of therapeutic gene expression by hUCBMC in lumbar spinal cord of ALS mice 4 weeks after cell transplantation. Double immunofluorescent staining demonstrates HNA^+-cells expressing VEGF, GDNF, or NCAM. *Upper panel* shows $HNA^+/VEGF^+$-cells, *middle panel*—$HNA^+/GDNF^+$-cells, and *lower panel*—$HNA^+/NCAM^+$-cells. Nuclei are counterstained with DAPI (*blue*). *Scale bar* 20 μm

molecule (NCAM1) was aimed to provide homing, migration, and survivability of the hUCBMC; glial-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF) played then role of neuroprotective agents. The strategy of triple gene therapy is under active investigation in Parkinson's disease. Stereotaxic intrastriatal injection of three adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I resulted in effective dopamine production in parkinsonian rats [13]. Herein, it is worth to mention cell-based gene delivery is safer in terms of viral immunity and the level of recombinant molecule production. Moreover, we believe the usage of hUCBMC for gene delivery results not only for expression of recombinant therapeutic genes. Grafted hUCBMC may be a source of biologically active molecules (cytokines, chemokines, growth and neurotrophic factors) and may give rise to different types of cells (more likely to endothelial and microglial cells). In our previous research, we demonstrated that transplantation of hUCBMC cotransduced with adenoviral vectors expressing GDNF and NCAM or VEGF and NCAM genes into ALS mice resulted in prominent increase of mice life span and performance in behavioral tests comparing to treatment with native hUCBMC or hUCBMC transduced with an adenoviral vector expressing VEGF or GDNF. In the present paper, we proved the usefulness for cotransduction of hUCBC with an Ad vector carrying gene of NCAM. The number of HNA^+-cells in lumbar spinal cord of ALS mice was higher in hUCBMC-VGN group at two studied time points. Mice from this group had better results in behavioral appearance and showed the longer period of life span. The data are related with our previous study and with results presenting that intramuscular transplantation of human mesenchymal stem cells engineered to simultaneously produce VEGF and GDNF had the synergistic effect on protection of neuromuscular junctions and survival of ALS rats [14]. Thus, the genetically engineered hUCBMC overexpressing

recombinant VEGF, GDNF, and NCAM is effective in ALS model and may have the potential as a new treatment option and candidate for further preclinical studies.

Acknowledgments This study was supported by the grant of Russian Science Foundation no. 16-15-00010. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities. Some of the experiments were conducted using the equipment of interdisciplinary center for collective use of Kazan Federal University supported by Ministry of Education of Russia (ID RFMEFI59414X0003), Interdisciplinary Center for Analytical Microscopy and Pharmaceutical Research and Education Center, Kazan (Volga Region) Federal University, Kazan,Russia.

References

1. Lim ST, Airavaara M, Harvey BK (2010) Viral vectors for neurotrophic factor delivery: a gene therapy approach for neurodegenerative diseases of the CNS. Pharmacol Res 61:14–26
2. McGinley LM, Sims E, Lunn JS, Kashlan ON, Chen KS, Bruno ES, Pacut CM, Hazel T, Johe K, Sakowsski SA, Feldman EL (2016) Human cortical neural stem cells expressing insulin-like growth factor-I: a novel cellular therapy for Alzheimer's disease. Stem Cells Transl Med 5:1–13
3. Kumagai G, Tsoulfas P, Toh S, McNiece I, Bramlett HM, Dalton WD (2013) Genetically modified mesenchymal stem cells (MSCs) promote axonal regeneration and prevent hypersensitivity after spinal cord injury. Exp Neurol 248:369–380
4. Jingzhong Z, Hui Y, Deyi D, Chunli D, Chunli Z, Xiaohong S, Jinlu Z, Qunyuan X (2005) Long-term therapeutic effects on parkinsonian rats of intrastriatal co-grafts with genetically engineered fibroblasts expressing tyrosine hydroxylase and glial cell line-derived neurotrophic factor. Int J Neurosci 115(6):769–779
5. Ou Y, Yu S, Kaneko Y, Tajiri N, Bae EC, Chheda SH, Stahl CE, Yang T, Fang L, Hu K, Borlongan CV, Yu G (2010) Intravenous infusion of GDNF gene-modified human umbilical cord blood CD34+ cells protects against cerebral ischemic injury in spontaneously hypertensive rats. Brain Res 1366:217–225
6. Iancu C, Ilie I, Mocan L, Georgescu C, Ilie R, Duncea I, Mocan T, Iancu D et al (2011) Human cord blood-derived stem cells in transplantation and regenerative medicine. Stem Cells in Clinic and Research. InTech. Available from: http://www.intechopen.com/books/stem-cells-in-clinic-and-research/humancordblood-derived-stem-cells-in-transplantation-and-regenerative-medicine
7. Ding DC, Chang YH, Shyu WC, Lin SZ (2015) Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. Cell Transplant 24(3):339–347
8. Islamov RR, Rizvanov AA, Mukhamedyarov MA, Salafutdinov II, Garanina EE, Fedotova VY, Solovyeva VV, Mukhamedshina YO, Safiullov ZZ, Izmailov AA, Guseva DS, Zefirov AL, Kiyasov AP, Palotás A (2015) Symptomatic improvement, increased life-span and sustained cell homing in amyotrophic lateral sclerosis after transplantation of human umbilical cord blood cells genetically modified with adeno-viral vectors expressing a neuro-protective factor and a neural cell adhesion molecule. Curr Gene Ther 15(3):266–276
9. Weydt P, Hong SY, Kliot M, Moller T (2003) Assessing disease onset and progression in the SOD1 mouse model of ALS. Neuroreport 14(7):1051–1054
10. O'Connor DM, Boulis NM (2012) Cellular and molecular approaches to motor neuron therapy in amyotrophic lateral sclerosis and spinal muscular atrophy. Neurosci Lett 527(2):78–84
11. Sun T, Ma QH (2013) Repairing neural injuries using human umbilical cord blood. Mol Neurobiol 47(3):938–945
12. Garbuzova-Davis S, Rodrigues MC, Mirtyl S, Turner S (2012) Multiple intravenous administrations of human umbilical cord blood cells benefit in a mouse model of ALS. PLoS One 7(2):e31254
13. Shen Y, Muramatsu SI, Ikeguchi K, Fujimoto KI, Fan DS, Ogawa M, Mizukami H, Urabe M, Kume A, Nagatsu I, Urano F, Suzuki T, Ichinose H, Nagatsu T, Monahan J, Nakano I (2004) Ozawa K. Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. Hum Gene Ther 11(11):1509–1519
14. Krakora D, Mulcrone P, Meyer M, Lewis C (2013) Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. Mol Ther 21(8):1602–1610