Biochemical and Biophysical Research Communications 451 (2014) 503–509

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Over-expression of *Oct4* and *Sox2* transcription factors enhances differentiation of human umbilical cord blood cells *in vivo*

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ARTICLE INFO

Article history:
Received 28 July 2014
Available online 11 August 2014

Keywords:
Amyotrophic lateral sclerosis
Differentiation
Umbilical cord blood mono-nuclear cell
Pluripotency
Transcription factor

ABSTRACT

Gene and cell-based therapies comprise innovative aspects of regenerative medicine. Even though stem cells represent a highly potential therapeutic strategy, their wide-spread exploitation is marred by ethical concerns, potential for malignant transformation and a plethora of other technical issues, largely restricting their use to experimental studies. Utilizing genetically modified human umbilical cord blood mono-nuclear cells (hUCB-MCs), this communication reports enhanced differentiation of transplants in a mouse model of amyotrophic lateral sclerosis (ALS). Over-expressing *Oct4* and *Sox2* induced production of neural marker PGP9.5, as well as transformation of hUCB-MCs into micro-glial and endothelial lines in ALS spinal cords. In addition to producing new nerve cells, providing degenerated areas with trophic factors and neo-vascularisation might prevent and even reverse progressive loss of moto-neurons and skeletal muscle paralysis.

1. Introduction

Regeneration is a fundamental feature of a living organism, responsible for the restoration of tissues during vital activity as well as after injures induced by endogenous and exogenous factors. Stimulation of the regeneration is inherently related to the life quality in patients with degenerative diseases, ischemic damages and traumatic injuries. Development of new technologies, such as gene and cell therapies, gave rise to the new findings, demonstrating the capability of these methods to stimulate the process of regeneration. Furthermore, preclinical trials and clinical application of the high technologies resulted from new discoveries in physics and chemistry have a great potential for the development of the regenerative medicine.

Organ allografting (for instance, heart, kidney or liver transplantation) is now accepted as a routine method in the clinical practice, while cell transplantation (bone marrow transplantation, peripheral and umbilical cord blood mononuclear cells transplantation) is mainly restricted to the therapy of blood disorders, for example, anaemia, hemoblastosis and cancer chemotherapy. Extensive research in the field of cellular therapy revealed its high efficiency in the stimulation of regeneration in the affected organs. However, advances in the field of stem cell therapy are mainly in the experimental studies because of ethical issues (the use of embryo and fetus derived cells), a risk of malignant transformation of transplanted stem cells, recipient infection with unknown pathogens from human donors and animal derived products used in cell culture, immune response to allogeneic cells. Thus, the cells subjected to the transplantation must have predictable and reproducible characteristics, namely – retain their viability; actively proliferate to produce a sufficient number of cells for the regeneration of injured tissues; be capable to integrate with host cells; differentiate into required cell types; restore a tissue structure and form a guide pathway for the regenerating axons; participate in re-myelination; produce the trophic and neuroprotective factors; stimulate neurite outgrowth and re-establishment of an intracellular contacts.

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http://dx.doi.org/10.1016/j.bbrc.2014.07.132
0006-291X/

Genetic modification of stem cells before transplantation is an innovative approach in the cell therapy and has a great potential for the development of the regenerative medicine. Expression vectors might be used for the target oriented migration of transplanted cells and production by them of a wide range of growth and trophic factors at the sites of degeneration. In addition to genetically modified cells, which express the therapeutic genes, much attention is paid to induced Pluripotent Stem cells – iPS cells. In the beginning of 2007, the peer reviewed journals published three independent studies, where the authors demonstrated the possibility to generate the iPS cells from the fibroblasts transfected with transcription factors Oct4, Sox2, cMyc, and Klf4 [1–3]. Obtained cells possess embryonic stem cell properties and after appropriate stimulation differentiate spontaneously into embryonic ectoderm, endoderm and mesoderm derivatives. Transplantation of iPS cells in mouse blastocyst results in generation of living chimaeras, which are able to produce germinal iPS-derived cells. In 2007, the next step was done towards the generation of iPS cells with therapeutic effect. Mouse iPS cells were successfully applied to the therapy of mouse sickle-cell disease [4]. Further, two independent research groups, Yamanaka and Tompson, have obtained iPS cells from human fibroblasts [5,6]. After this Dimos et al. has generated iPS cells from 82-old woman skin fibroblasts diagnosed with amyotrophic lateral sclerosis [7]. In this study, obtained iPS cells were differentiated into cholinergic neurons. Recently, another group of scientists demonstrated generation of iPS cells from ALS patients by both integration and transgene-free delivery methods of reprogramming transcription factors and differentiation them into motor neurons [8].

Classic technologies of non-germline cell de-differentiation use the retroviral gene transfer system. Viral transduction, however, might result in insertional mutagenesis following cell malignant transformation. Therefore, there is a need for development of safe methods for non-viral genetic delivery of genetic information into the cells. One of them – the use of plasmid vectors.

Type of cells subjected to de-differentiation is an important issue for the cell therapy. Genetic modification of stem cells is widely used in experiments for therapeutic gene delivery into the site of injury, and, particularly, by using human umbilical cord blood cells. These cells were recently shown to be able to differentiate into neuron like cells upon incubation in specific medium, expressing different MAPKs, apoptosis, and neurogenesis markers and downregulation in the cell proliferation markers during neuronal differentiation [9]. Blood and bone marrow mononuclear cells compose of different types of cells, including progenitor and stem cells, possessing capacity for extended differentiation after modification with transcription factors regulating their pluripotency. Thus, genetic modification of human umbilical cord blood cells might be important in regard to two issues. Firstly, for the delivery of specific growth and trophic factors to support cell viability under degenerative conditions with different etiology. And secondly, for the de-differentiation of stem cells into iPS-like cells following their directed differentiation into required cell types. In this study we investigated phenotype profiles of mononuclear cells from human umbilical cord blood transfected with plasmid vectors expressing transcription factors Oct4 and Sox2 after transplantation into ALS mice.

2. Materials and methods

2.1. Plasmid constructs generation

Cloning of human Sox2 and Oct4 genes was performed in several steps. First, we RT-PCR amplified cDNA of corresponding genes using specific primers (Table 1) and PrimeSTART DNA polymerase (Takara). 3′ adenosines were added to PCR products by incubation with GoTaq polymerase (Promega) and purified PCR products were TA-cloned into pGEM Teasy vector system (Promega, USA) according to manufacturer's instructions. cDNA from resulting pGEM-Sox2 and pGEM-Oct4 plasmids were subcloned into pBudCE4.1 Mammalian Expression Vector (Life Technologies) using KpnI/XhoI and SalI/XbaI restriction enzymes (Promega) correspondingly. Successful subcloning of cNDAs was confirmed by sequencing. As a control we used pBud-EGFP plasmid, described previously [10]. Preparative quantities of plasmid DNA were purified using Plasmid Maxi Kit (QIAGEN).

2.2. Cell preparation

Mononuclear fraction of human UCB was isolated by standard technique of sedimentation on to a density barrier (Ficoll, 1.077 g/ml) as described previously [11]. Isolated cells were transiently transfected by electroporation (Gene Pulser Xcell Total System, BioRad, 20 ug DNA, 0.4 cm cuvette, 300V, 1000 uF) with either control plasmid (pBud-EGFP) or expression plasmid (pBud-Sox2-Oct4) as described previously [10]. Cells were allowed to recover for 24 h in RPMI1640 media containing 10% FBS. Thereafter 1×10^6 cells were injected retro-orbitally in presymptomatic B6SJL-TG(SOD1-G93A)^{dl}1Gur/J mice (The Jackson Laboratory, USA).

2.3. Transcription factors mRNA expression analysis in transfected UCB-MCs using real-time PCR

Expression level of human pluripotency associated transcription factors (Oct4, Sox2, Klf4, cMyc and NANOG) mRNA in UCB-MCs was examined by real-time PCR 48 h post transfection using iQ5 Multicolor Real-Time PCR Detection system (BioRad, USA) as described previously [12]. Total RNA from cell cultures was isolated using HighPure RNA Isolation Kit (Roche, UK) according to the manufacturer's instructions. Embryonic stem cells (ESCs) mRNA from human embryonic stem cell line Moscow 01 (hESM01) was kindly provided by Dr. Sergey L. Kiselev (Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow). cDNA synthesis was performed utilizing random hexamer primers and moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Promega, Madison, WI, USA) at 37 °C for 1 h. Real-time PCR primers and TaqMan probes, as well as 2.5× premix for TaqMan real-time PCR was purchased from Syntol (Moscow, Russia) and used according to manufacturer's instructions. Amount of RNA was normalized using β-actin. Serial dilutions of cDNA synthesized from mRNA of ESC was used to generate standard curve for relative quantitation of gene-expression. Expression level of genes in ESCs was considered as 100%. PCR were run in duplicates. Standard error and *t*-test values were obtained using Microsoft Excel 2007, with $P < 0.05$ being regarded as a statistically significant difference.

Table 1
Primers used for PCR amplification of specific genes.

Name	Oligonucleotide sequence	Application
hSOX2-ORFF-KpnI	CACgGtaCCacCATGTACAACATGATGG	RT-PCR, forward primer
hSOX2-ORFR-Stop-XhoI	TCCtcgagTCACATGTGTGAGAGGGGCAG	RT-PCR, reverse primer
hOct4v1-ORFR-Stop-XbaI-EcoRI	CCCCATTCCTAGAgaattctctAgaTCAGTTTGAATGC	RT-PCR, forward primer
hOct4v1-ORFF-BamHI-SalI	GGGgatCcgtCgaCaCCATGGCGGGA	RT-PCR, reverse primer

2.4. Animals and treatments

Transgenic for *hSOD1* mice (B6SJL-TG(SOD1-G93A)^{dl}1Gur/J), were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and bred at animal facility of Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (Russian Academy of Science). Mature 21-week old mice were delivered to Kazan State Medical University. Mice were housed one per cage, under standard laboratory conditions, with a 12-h light/dark schedule and unlimited access to food and water. Transgenic female and male mice were randomly assigned to experimental groups according transplantation of genetically modified hUCB-MC cells expressing: (1) EGFP ($n = 4$) and (2) Oct-Sox ($n = 4$). In all groups 1×10^6 cells were injected retro-orbitally in pre-symptomatic 27-week old G93A mice. Animal protocol was approved by the Kazan State Medical University Animal Care and Use Committee.

2.5. Immunofluorescence

Two and four weeks after transplantation the mice were anesthetized by intraperitoneal injection of 16% sodium pentobarbital solution and were transcardially perfused first with cold phosphate buffered saline (PBS) and then with cold 4% paraformaldehyde in PBS (pH 7.4). The whole spinal cord was removed, post-fixed with paraformaldehyde overnight at 4 °C and then immersed in 15% sucrose solution in 0.1 M cacodylate buffer, pH 7.3, for 1 day at 4 °C as described before [13]. The spinal cords were subdivided into cervical, thoracic, and lumbar parts. Afterwards, the tissue was frozen for 2 min in 2-methyl-butane (isopentane) precooled to −30 °C. For sectioning the spinal cord segments were attached to a cryostat specimen holder using TissueTek® (Sakura Finetek Europe, Zoeterwoude, The Netherlands). Serial longitudinal sections of 20 μm thickness were cut on a cryostat HM560 Cryo-Star (Carl Zeiss) and picked up on SuperFrost®Plus glass slides. To detect transplanted cells in the host tissue and to identify their phenotype, spinal cord crysections were subjected to a double immunofluorescent staining. For all sections antigen retrieval was performed by immersion into 0.01 M sodium citrate solution, pH 9.0, heated to 70 °C in a water bath for 30 min. After cooling of the slides to RT, blocking of non-specific binding sites was performed using PBS containing 0.2% Triton X-100, 0.02% sodium azide and 5% normal goat serum for 1 h at RT. For a double immunostaining the cocktail containing primary antibodies (Table 2) were applied for 3 days at 4 °C following washing in PBS (3×15 min at RT) and incubation with donkey anti-mouse IgG conjugated with DyLight549 and donkey anti-rabbit or donkey anti-chicken IgG conjugated with DyLight649 (Jackson ImmunoResearch Laboratories, dilution 1:200) for 2 h at RT.

After a subsequent washing in PBS cell nuclei were stained for 10 min at RT with bis-benzimide solution, washed again in PBS, mounted in anti-quenching medium and examined with a laser scanning microscopy (Zeiss Axiovert 200 M).

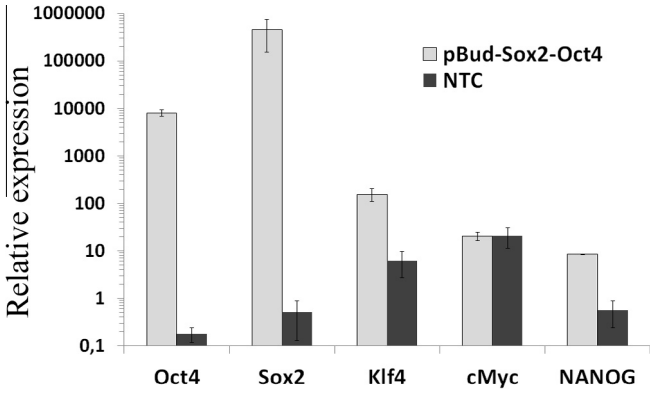

Fig. 1. Real-time PCR analysis of mRNAs expression level for human pluripotency associated transcription factor (Oct4, Sox2, Klf4, cMyc, NANOG) in hUCB-MC electroporated with plasmid pBud-Sox2-Oct4 or non-transfected control (NTC). Expression level of mRNA for transcription factors in ESC was considered as 100%.

3. Results

3.1. Expression of recombinant genes in vitro

UCB mononuclear cell fraction is reach of different immature cells. There are hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), unrestricted somatic stem cells (USSCs), cord blood-derived embryonic stem cells (CBES), side population cells (SP), and endothelial progenitor cells [14]. Based on this data we have maintained pluripotency in UCB-MCs via upregulation of Oct3/4 and Sox2 expression.

Real-time PCR expression analysis revealed high level of mRNA expression for Klf4 and cMyc in hUCB-MCs compared to ESC (Fig. 1). Expression level of NANOG mRNA was lower than in ESC, however still significantly. At the same time expression level of Sox2 and Oct4 mRNA in hUCB-MCs was significantly lower compared to ESC.

To increase expression level of recombinant Sox2 and Oct4 endogenously, hUCB-MCs were transfected with genetic construct, expressing these transcription factors genes, Dual expression cassette plasmid pBud-Sox2-Oct4 was generated using standard genetic engineering techniques utilizing the plasmid backbone pBudCE4.1. As a transfection control we used previously generated plasmid pBud-EGFP. Efficient expression of EGFP in UCB-MCs after electroporation with pBud-EGFP was confirmed by fluorescent microscopy (data not shown). Recombinant plasmid pBud-Sox2-Oct4 was constructed, which carries 2 transcription factors genes, Sox2 and Oct4, under the control of 2 independent constituently active promoters, thus resulting in efficient simultaneous and independent expression of recombinant proteins. and subjected

Table 2
Primary antibodies used in this study.

Antibody	Cellular phenotypes/structures recognized	Host	Code/clone	Dilution	Source
HNA (Human nuclei antigene)	Nuclei of human cells	Mouse	MAB1281	1:20	Chemicon®
Ki67	Nuclei of proliferating cells	Rabbit	ab5580	1:500	Abcam
PGP9.5 (Ubiquitin Hydrolase)	Neurons	Chicken	200-901-E52	1:500	Rockland
CD34	Endothelial cells	Rabbit	ab81289	1:100	Abcam
S100	Astrocytes	Rabbit	Z0311	1:2,000	DakoCytomation
OSP (Oligodendrocyte Specific Protein)	Oligodendrocytes	Rabbit	ab53041	1:2,000	Abcam
Iba1	Microglia	Rabbit	131 011	1:600	Biocare Medical

to real-time PCR analysis. Significant increase in the mRNA expression level was achieved in transfected hUCB-MC compared to un-transfected control cells. We observed a significant increase in Sox2 mRNA (1×10^5 times) and Oct4 mRNA (4×10^3 times) after electroporation of UCB-MCs with plasmid pBud-Sox2-Oct4 (Fig. 1). Interestingly, expression of recombinant Sox2 and Oct4 genes in genetically modified UCB-MCs resulted in elevated expression of endogenous Klf4 and NANOG mRNAs indicating a potential reprogramming processes in these cells. Expression of cMyc mRNA was not significantly affected by transfection of UCB-MCs with plasmid pBud-Sox2-Oct4. This might be explained by the high endogenous level of cMyc mRNA in un-transfected UCB-MCs (Fig. 1).

It is very well known that Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc) and their core developmental signaling network is necessary for pluripotency [2]. Previous studies have demonstrated the function of both Oct3/4 and Sox2 as core transcription factors to determine pluripotency [15]. Comparative analysis of regulatory elements of genes has identified a conserved Oct3/4 and Sox2 co-binding domains in most embryonic stem cells expressed genes, highlighting the importance of this transcriptional pathway. It was suggested that that Oct3/4 and Sox2 cooperatively activate or repress a set of genes (including *Oct3/4*, *Sox2* and *Nanog*) through their Oct-Sox enhancers. This, in turn, results in their cooperative regulation of additional downstream genes.

3.2. Phenotypic characteristics of UCB-MCs genetically modified to overexpress Sox2 and Oct4

Immunofluorescent analysis of lumbar spinal cord two and four weeks after retro-orbital injection of native and genetically modified UCB-MCs revealed human nuclei antigen (HNA)-positive cells in both, gray and white matter. In the spinal cords of experimental animals we observed a higher number of transplanted pBud-Sox2-Oct4 transfected UCB-MCs as compared to spinal cords of mice subjected to the transplantation with native, non-transfected UCB-MCs. Double immunofluorescent analysis with Ab against HNA and S100 (specific marker for astrocytes) or with Ab against HNA and OSP (oligodendrocyte specific protein) revealed $HNA^+/S100^-$ UCB-MCs in gray matter surrounded by $S100^+$ cells (Fig. 2A) and HNA^+/OSP^- UCB-MCs in white matter appearing between OSP^+ cells (Fig. 2B). Interestingly, some UCB-MCs were observed in blood vessels of spinal cord two weeks after transplantation (Fig. 3A). The presence of HNA^+ UCB-MCs circulating in blood stream illustrates their high migration potential with following addressed homing of UCB-MC from blood into CNS. We have also detected UCB-MCs expressing the marker of endothelial cells – CD34 ($HNA^+/CD34^+$ UCB-MCs, Fig. 3B). Noteworthy that endothelial cells differentiated from UCB-MCs were found in spinal cord blood vessels endothelium (Fig. 3C).

Immunofluorescent analysis with Ab against HNA and Iba1 (specific marker for microglial cells) indicated $HNA^+/Iba1^+$ UCB-MCs (Fig. 4A). Iba1 expression profile revealed cytoplasmic and nuclear localization in native as well as in newly differentiated microglial cells from UCB-MC. Some UCB-MCs were close located to native Iba^+ cells (Fig. 4B). We have also observed UCB-MCs surrounded by Iba^+ cells suggesting partial ingestion of transplanted cells by microglial cells (Fig. 4C). These data demonstrate the natural differentiation of monocytes and endothelial progenitor cells from UCB-MCs into functional mature microglial and endothelial cells.

Double immunofluorescent analysis with Ab against HNA and Ki67 (specific marker for proliferating cells) revealed $HNA^+/Ki67^+$ UCB-MCs (Fig. 5A). The finding of dividing pBud-Sox2-Oct4 transfected UCB-MC in spinal cord demonstrates the proliferating activity of UCB-MCs in spinal cord parenchyma. The intriguing data were obtained with double immunofluorescent analysis with Ab against HNA and PGP9.5 (specific marker for neural cells). In spinal cord of mice injected with pBud-Sox2-Oct4 transfected UCB-MCs we detected $HNA^+/PGP9.5^+$ UCB-MC (Fig. 5B). These results are in agreement with previously published data demonstrating an expression of neural differentiation markers by UCB-MCs transplanted into ALS mice [16].

4. Discussion

Application of human embryonic stem cells in regenerative medicine is controversial. Cellular differentiation processes during normal embryonic development are guided by extracellular soluble factors such as morphogen gradients and cell contact signals,

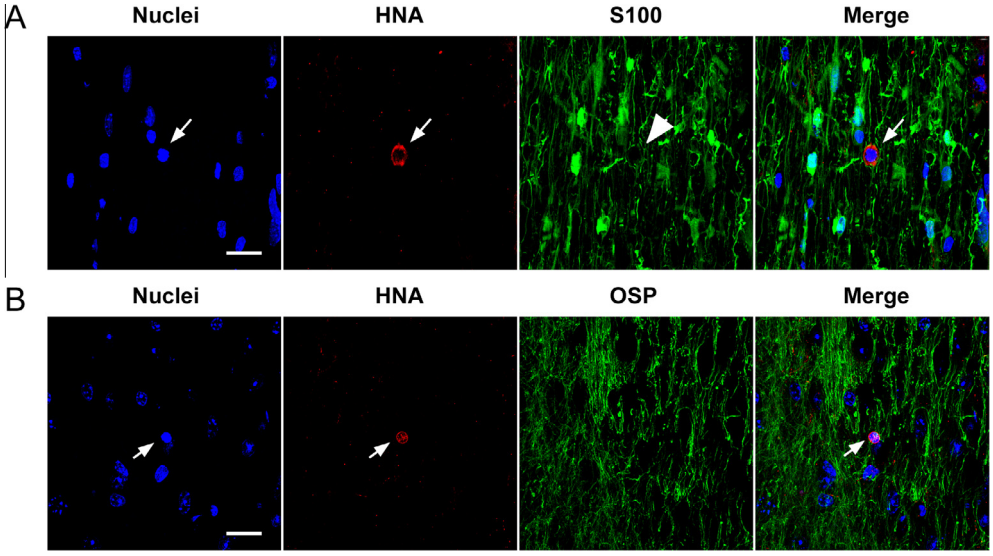

Fig. 2. pBud-Sox2-Oct4 transfected UCB-MCs in the spinal cord of G93A mice after transplantation. (A) No co-localization of human nuclei antigen (HNA, red) and S100 (green) is observed. Arrows on panels show $HNA^+/S100^-$ cell, arrowhead indicates $S100^+$ cell processes surrounding $HNA^+/S100^-$ cell. Scale bar: 25 μm. (B) pBud-Sox2-Oct4 transfected UCB-MC (HNA, red) do not express Oligodendrocyte Specific Protein (OSP, green) after transplantation into spinal cord of G93A mice. Arrows on panels show HNA^+/OSP^- cell. Scale bar: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

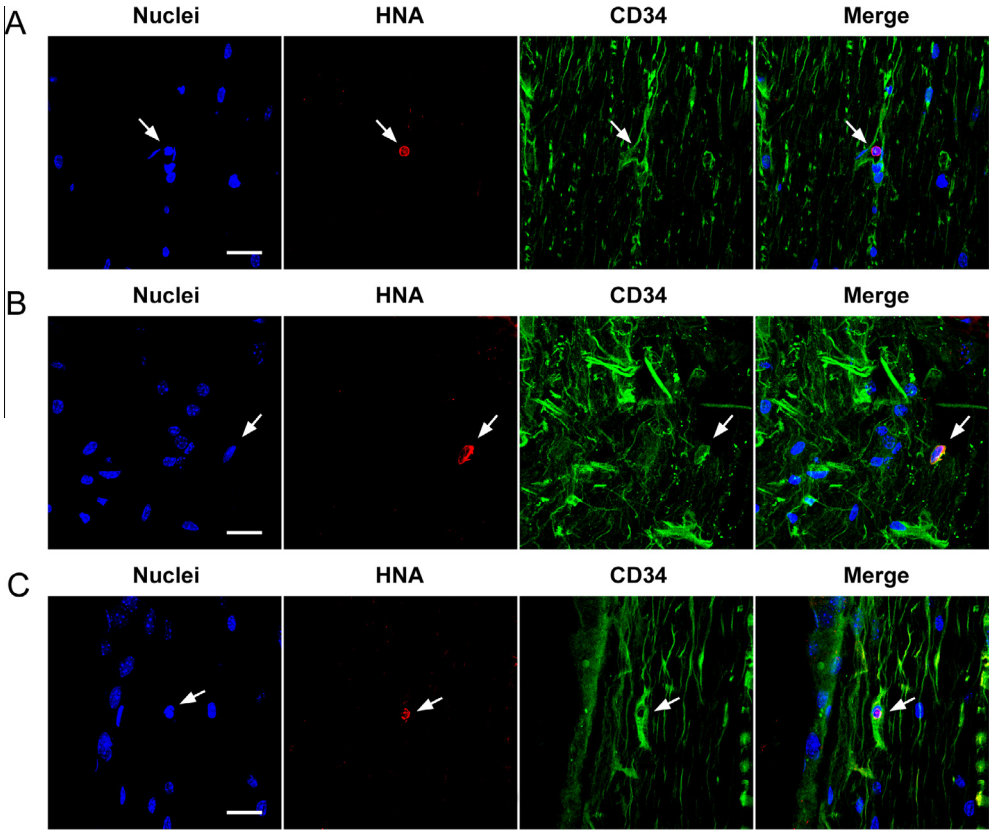

Fig. 3. pBud-Sox2-Oct4 transfected UCB-MCs (HNA, red) after transplantation into G93A mice. Expression of CD34, marker for endothelial cells, is shown in green. (A) UCB-MCs in blood vessel of spinal cord is shown. Arrows on panels depict HNA^+ in blood vessel. Scale bar: 25 μm. (B and C) Expression of CD34 by pBud-Sox2-Oct4 transfected UCB-MCs (HNA, red). Arrows on panels indicate $HNA^+/CD34^+$ cells. Scale bar: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which might result in induction of specific combinations of lineage-determining transcription factors [17]. To induce stem cell differentiation in the required direction, the stem-cell biology has created reprogramming technology. Thus, somatic cells can be reprogrammed by transferring their nuclear contents into oocytes [18] or by fusion with embryonic stem cells [19]. These cells contain factors which have been shown to confer pluripotency to somatic cells. Among them Oct3/4 [20], Sox2 [21], and Nanog [22], maintain the pluripotency in both early embryos and embryonic stem cells. Genes such as *Stat3* [23], *E-Ras* [24], *c-myc* [25], *Klf4* [26], and *β-catenin* [27] have been shown to contribute the long-term maintenance of the embryonic stem cell phenotype and the rapid proliferation of embryonic stem cells in culture. Studies with embryonic stem cells have demonstrated that the Oct4, Sox2, Klf4 and c-Myc – the 'Yamanaka factors' – also named as 'OSKM' factors are able to generate induced pluripotent stem (iPS) cells from different cell types, and their role in cell reprogramming is now well established [5]. Furthermore, this finding was confirmed by observation that somatic cells can be reprogrammed to generate induced pluripotent stem cells (iPSCs) by overexpression of four transcription factors, Oct4, Klf4, Sox2, and c-Myc, indicating them as a mediators of the pluripotent status in adult somatic cells. These transcription factors maintain pluripotency by decreasing expression of differentiation-associated genes and increasing expression of "stemness" genes. Inner cell mass of mammalian blastocysts and derived from them embryonic stem cells are characterized to maintain pluripotency and upregulated expression of Oct3/4 and Sox2. However, the ability to restore pluripotency in adult somatic cells through the ectopic co-expression of Oct3/4 and Sox2 needs the co-expression of c-Myc and Klf4 which modify chromatin structure so that Oct3/4 and Sox2 can bind to their targets [5,28].

Transcription factors play a crucial role in the fate of stem cells in regarding their neuronal differentiation. As reviewed by Ang et al. and Yang et al. [17,29], induction of neuronal reprogramming might be mediated by a pool of 19 candidate transcription factors, those certain combination revealed the most promising results. Thus, defined combinations of transcription factors such as pro-neural transcription factor *Mash1* (also known as *Ascl1*), *Nurr1* (also known as *Nr4a2*), *Lmx1a*, *Brn2* (also called *Pou3f2*), *Myt1l*, *NeuroD1*, and *FoxA2* for direct trans-differentiation of human fibroblasts into neurons have been emerged as a powerful approach for both disease modeling and repair [30,31]. Other transcription factors were also shown to play a critical role in maintaining embryonic stem cell pluripotency and neuronal fate of embryonic stem cells. Thus, earlier observations have demonstrated that sustained upregulation of Oct3/4 in embryonic stem cells leads to efficient neuroectoderm formation and subsequent neuronal differentiation [32]. Recently, neuronal stem cells were generated by transfection of adult human peripheral $CD34^+$ cells with Sox2, Oct3/4, c-Myc and Klf4, and these cells could be differentiated to glial cells and action potential firing neurons [33]. Similar results were shown with reprogramming of mouse Lewis lung carcinoma transfected with the same transcription factors [34]. In this study, reprogramming of cancer cells into pluripotent stem cells induced functional pluripotency of the reprogrammed cells confirmed by their ability to form embryoid bodies and differentiate into neuronal progenitors on retinoic acid treatment [34].

Amyotrophic lateral sclerosis is a devastating neurodegenerative disease, characterized by progressive loss of brain

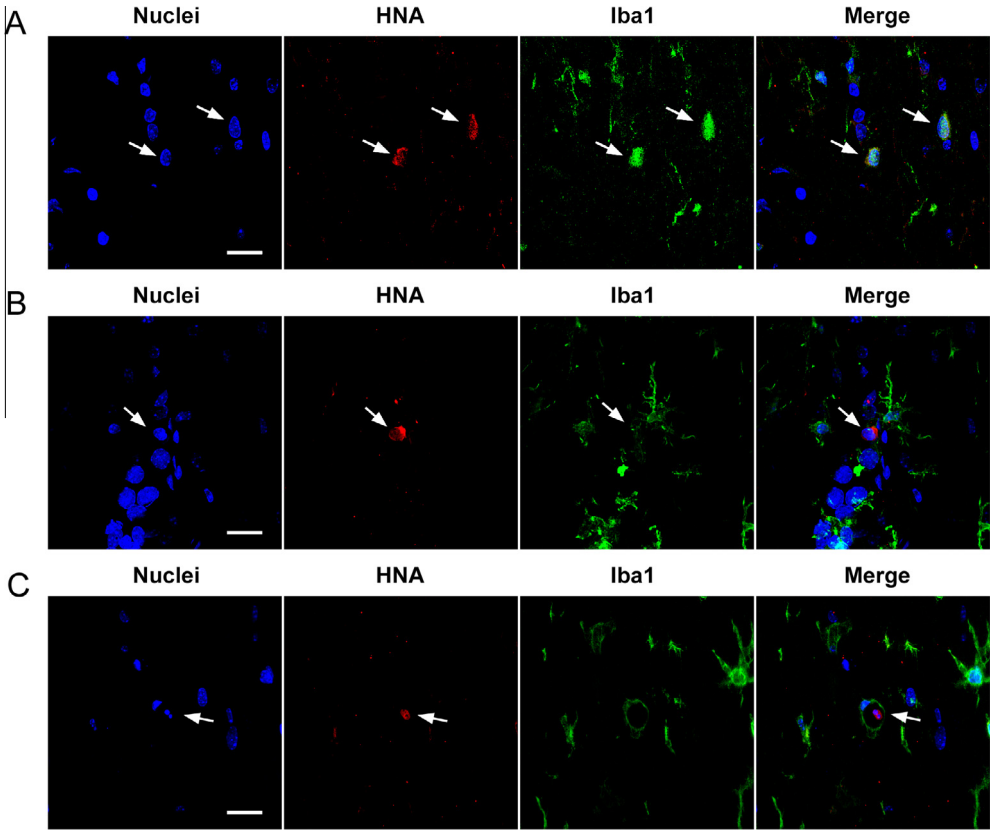

Fig. 4. pBud-Sox2-Oct4 transfected UCB-MCs (HNA, red) revealed an expression of microglial marker Iba1 (green) after transplantation into spinal cord of G93A mice (A). Arrows on panels indicate $HNA^+/Iba1^+$ cells. Scale bar: 25 μm. (B) UCB-MCs (HNA, red) neighboring to $Iba1^+$ microglial cells. Arrows on panels indicate $HNA^+/Iba1^-$ cell. Scale bar: 25 μm. (C) UCB-MCs (HNA, red) ingested by $Iba1^+$ cell. Arrows on panels indicate HNA^+ cell. Scale bar: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

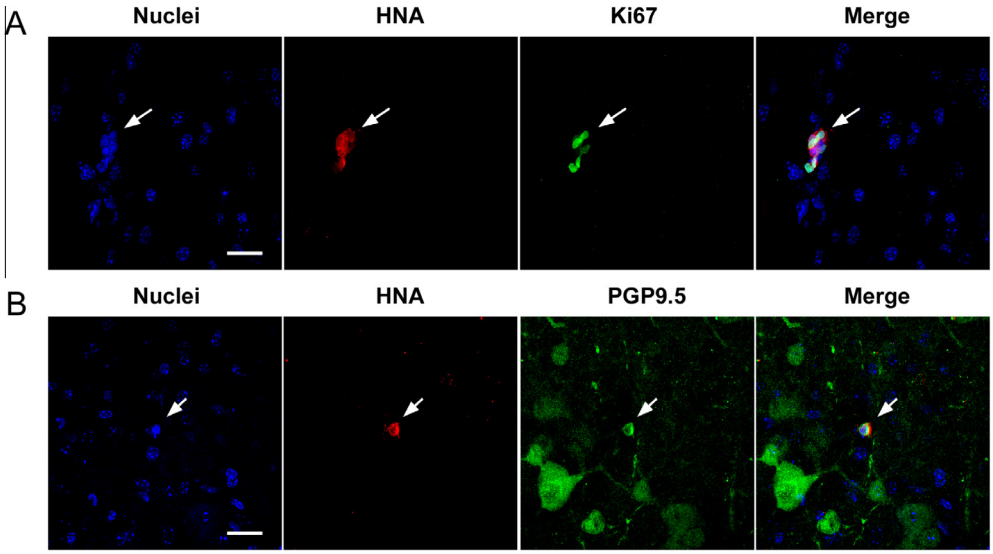

Fig. 5. Dividing pBud-Sox2-Oct4 transfected UCB-MC (A) after transplantation into spinal cord of G93A mice. Co-localization of human nuclei antigen (HNA, red) and Ki67 (specific marker for proliferating cells, green) is shown. Arrows on panels depict $HNA^+/Ki67^+$ cells. Scale bar: 25 μm. (B) Expression of the neural marker PGP9.5 (green) by UCB-MCs. Co-localization of human nuclei antigen (HNA, red) and PGP9.5 (specific marker for neural cells) is shown. Arrows on panels depict $HNA^+/PGP9.5^+$ cells. Scale bar: 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and spinal cord motor neurons, resulting in skeletal muscle paralysis throughout the body and ultimately leading to patient's death within 3–5 years after onset of paralytic symptoms due to respiratory failure. Replacement of lost neurons, stimulation of neuritis growth and reestablishment of neuronal circuits are anticipated results in effective treatment of ALS. In our previous studies we investigated VEGF-L1 and VEGF-FGF2 gene-modified UCB-MCs for ALS treatment in ALS mice [10,11]. We have shown that

UCB-MC overexpressing VEGF-L1CAM had increased homing and differentiation into endothelial cells, while UCB-MCs overexpressing VEGF-FGF2 may differentiate into astrocyte. In the present study, for ALS treatment we used UCB-MCs overexpressing transcription factors related to pluripotency, Oct4 and Sox2. We have demonstrated that UCB-MCs transfected with pBud-Sox2-Oct4 plasmid DNA increased their survivability, migration potential, homing, proliferation, and possibility to differentiate into endothelial cells, microglial cells and, importantly, in neural cells. Although some Oct4-Sox2 overexpressing UCB-MCs stayed undifferentiated and did not express any studied markers, the fact that Oct4-Sox2 transfected UCB-MCs may express neuronal marker suggests the possible replacement of dying spinal motor neurons. It would be therefore interesting to analyze in future studies the ability of newly formed neurons to re-establish neural circuits in spinal cord and send axons to the target skeletal muscles. At the same time we showed that UCB-MCs over-expressing Oct4 and Sox2 have an ability to differentiate into different cell types of nervous tissue such as endothelial and microglial cells. Taken together, results of our study indicate a novel method to induce pluripotency in UCB-MCs using transcriptional factors Oct4 and Sox2. This method is biologically much safer comparing with iPS and cell products obtained from iPS.

Statement of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Contributors

All authors contributed in the conception and design, or experiments, analysis and interpretation of the study data, and participated in drafting of the manuscript or revising it critically for important intellectual content. All authors have read and gave final approval of the manuscript and have no conflict of interests to disclose.

Acknowledgments

The study was supported by Russian Science Foundation grants #13-04-97156 and #14-15-00847, by the Program of Competitive Growth of Kazan Federal University, and by *Asklepios-Med* (Hungary). Some of the experiments were conducted with support of Federal Center of Collective Use and Pharmaceutical Research and Education Center, Kazan (Volga Region) Federal University, Kazan, Russia.

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