Cell and Tissue Research (2019) 376:179–187
https://doi.org/10.1007/s00441-018-02980-x

REGULAR ARTICLE

Dental pulp stem cell transplantation ameliorates motor function and prevents cerebellar atrophy in rat model of cerebellar ataxia

Abbas Aliaghaei[1] · Mahdi Eskandarian Boroujeni[2] · Houssein Ahmadi[1] · Amir-Hossein Bayat[3] · Mostafa Rezaei Tavirani[4] · Mohammad Amin Abdollahifar[1] · Mohammad H. Pooyafar[5] · Vahid Mansouri[6]

Received: 13 July 2018 / Accepted: 13 December 2018 / Published online: 12 January 2019

Abstract

Cerebellar ataxias (CA) include a range of neurodegenerative disorders hallmarked by deterioration of the cerebellum. Cell replacement therapy (CRT) offers a potential remedy for the diseases associated with the central nervous system (CNS). This study was designed to assess the neurorestorative/protective effects of dental pulp stem cell (DPSC) implantation on a rat model of CA induced by 3-acetylpyridine (3-AP) as a neurotoxin. To begin, human DPSCs were extracted, cultured and phenotypically characterized. Then, experimental ataxia was induced in 20 male adult rats by a single injection of 3-AP and bilateral DPSC transplantation was performed 3 days after 3-AP administration, followed by stereological analysis of cerebellar layers along with assessment of motor skills and inflammatory response. The findings showed that transplantation of DPSCs in a 3-AP model of ataxia ameliorated motor coordination and muscle activity, increased cerebellar volumes of molecular and granular layers plus white matter, reduced the levels of inflammatory cytokines and thwarted the degeneration of Purkinje cells against 3-AP toxicity. Taken together, human DPSCs could be considered as a suitable candidate for CRT-based therapies with a specific focus on CA.

Keywords Cerebral ataxia · Dental pulp stem cells · Motor activity · Cerebellar volume · 3-Acetylpyridine

Abbas Aliaghaei and Mahdi Eskandarian Boroujeni contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00441-018-02980-x) contains supplementary material, which is available to authorized users.

✉ Vahid Mansouri
vm1343@yahoo.com

Abbas Aliaghaei
aghaei60@gmail.com

Mahdi Eskandarian Boroujeni
esmahdee@gmail.com

Houssein Ahmadi
hosseinahmadi9213@yahoo.com

Amir-Hossein Bayat
ah.bayat@yahoo.com

Mostafa Rezaei Tavirani
tavirani@yahoo.com

Mohammad Amin Abdollahifar
m_amin58@yahoo.com

Mohammad H. Pooyafar
mhpooyafar167@gmail.com

[1] Cell Biology & Anatomical Sciences Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Department of Stem Cells & Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering & Biotechnology, Tehran, Iran

[3] Department of Neurobiology and Neuropsychology, Saveh University of Medical Sciences, Saveh, Iran

[4] Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[5] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[6] Faculty of Paramedical Science, Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

Cerebellar ataxias (CA) include a range of neurodegenerative disorders hallmarked by deterioration of the cerebellum along with a host of neurological symptoms (Brusse et al. 2007; Marmolino and Manto 2010). Individuals with CA exhibit pathophysiological conditions including dyssynergia, dysmetria, nystagmus, postural sway and dysarthria (Marsden and Harris 2011). Cell replacement therapy (CRT) offers a potential remedy for the diseases associated with the central nervous system (CNS) (Boroujeni and Gardaneh 2017). Among various cell candidates, dental pulp–derived adult mesenchymal stem cells (MSCs) are considered suitable for CRT due to their invaluable properties such as immunomodulatory and multi-differentiative potentialities (Gronthos et al. 2002; Miura et al. 2003; Wang et al. 2012). Moreover, dental pulp stem cells (DPSCs) disclosed efficient neurogenic differentiation as well as superior neuroprotection compared with bone marrow–derived MSC (BM-MSC) in animal models of neurodegenerative diseases (Govindasamy et al. 2010; Ibarretxe et al. 2012; Sakai et al. 2012; Yamagata et al. 2013). In view of the neural crest origin of DPSCs, the neurotrophic expression levels of factors including the brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNF) and glial-derived neurotrophic factor (GDNF) appeared to be higher in contrast to BM-MSC (Sakai et al. 2012). A single dose of 3-acetylpyridine (3-AP) generates, within 24 h of administration, symptoms of CA and destruction to the medulla oblongata and to the climbing fibers of the cerebellum (Butterworth et al. 1978). Additionally, the neurotoxin 3-AP triggers selective degeneration of neurons of inferior olive nucleus in rodents. A single inferior olive axon in rats divides a number of climbing fibers that end in cerebellar regions such as molecular and granular layers. Hence, degeneration of nerve fibers innervating the cerebellum by 3-AP provokes a pattern of neuronal loss similar to the typical features of human CA (Janahmadi et al. 2009; Sierra et al. 2003). Besides, after 3-AP administration, rats exhibit evident motor deficits with staggering of their hind limbs and gait abnormalities (Fernandez et al. 1998). However, this 3-AP model might not accurately represent human disease but the rationale for using this model is because the neurotoxin 3-AP selectively degenerates calbindin-positive neurons in the inferior olive and this nucleus has a crucial role in the modulation of the cerebellar function. Further, this procedure is used in many investigations to examine CA such as Jiang et al. (2015).

In this study, we seek to investigate the neurorestorative/protective effects of DPSC implantation on a rat model of CA induced by 3-AP.

Materials and methods

Isolation and culture of DPSCs

Human-impacted third molars were obtained with informed written consent. Human dental pulp explants were obtained from partially or completely impacted third molar teeth of five patients, aged 25–30 years. The dental pulp tissue fragments were minced using sterilized scalpels and digested in collagenase type I 3 mg/ml (Invitrogen) for 1 h at 37 °C. Once digestion was completed, the obtained cell pellet was suspended in 600 μl of PBS and was passed through a 100-μm cell strainer. The isolated dental pulp stem cells (DPSCs) were cultured in Dulbecco's modified Eagle medium (DMEM)/F-12 1:1 supplemented with L-glutamine and 10% fetal bovine serum (FBS) and incubated in a humidified chamber at 37 °C and 5% CO_2.

Flow cytometric analysis

DPSCs were detached with 0.25% trypsin; the samples were centrifuged; nearly 5×10^5 cells were re-suspended in 10% FBS and then reserved on ice packs for 10 min. The cells incubated with 10% serum at 4 °C for 1 h. Next, the serum was removed and the cells were incubated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-labeled monoclonal antibodies against human surface markers at 4 °C for 1 h. CD90 and CD105 were used against mesenchymal stem cell markers. CD45 was used against the hematopoietic marker. Isotype antibodies were also used as control samples.

Osteogenic and adipogenic differentiation

At 70–80% confluence, the medium was changed with osteogenic medium composed of DMEM/F12 complemented with 10% FBS, 50 μg/ml ascorbic 2-phosphate, 10 nM dexamethasone and 10 mM β-glycerol phosphate. After approximately 21 days, the cells were fixed in 4% paraformaldehyde and then stained with 2% Alizarin Red solution for 20 min to detect the formation of mineralized crystals in extracellular matrix. For adipogenic differentiation, cell medium was substituted with adipogenic medium composed of DMED/F12 supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 50 μM indomethacin (Sigma, USA) and 10 nM dexamethasone. The medium was changed every 3 days. After 15 days of incubation, the cells were fixed in 4% paraformaldehyde and then prepared for staining with 0.5% Oil Red O solution for 30 min. Formation of multiple lipid droplets inside the mesodermal stem cells was photographed by a phase contrast microscope.

Experimental model of CA in rats

In this study, 30 adult male rats (Sprague–Dawley, 200–220 g) were obtained from the Laboratory Animal Center of Shahid Beheshti University Of Medical Sciences, Tehran, Iran. Rats were housed at 22 °C, under 12-h light/12-h dark conditions with ad libitum access to food and water. Rats were randomly assigned into three groups: control group ($n = 10$), 3-AP + vehicle group ($n = 10$) and 3-AP + DPSCs group ($n = 10$). In the 3-AP groups, all animals received an intraperitoneal (i.p.) single dose of 3-AP injections (75 mg/kg). The timeframe of our experimental design is shown in Supplementary Fig. 1.

Western blot

For detection of neurotrophic factors of VEGF and GDNF, DPSCs were lysed in lysis buffer containing a protease inhibitor. Twenty microgram proteins were loaded on 12% SDS-PAGE gel, electrophoresed and then transferred to PVDF. The membrane was incubated with a blocking solution for 75 min. Afterwards, the blots were incubated with primary antibodies VEGF and GDNF at 4 °C. Then, blots were washed and incubated with secondary antibody for 90 min. Immunoreactive polypeptides were detected using ECL reagents and autoradiography.

Stem cell transplantation

Bilateral DPSC transplantation was performed 3 days after 3-AP administration. The animals were assigned to one of the three experimental/transplant groups: control or intact group ($n = 10$), 3AP + vehicle ($n = 10$) and 3-AP + DPSCs (300,000 cells; $n = 10$). The DPSCs were maintained alive in suspension containing 2 μl DMEM/F12 aliquot, stored in ice during the surgery procedure. After being anesthetized intraperitoneally with xylazine (10 mg/kg)/ketamine (75 mg/kg), the animals were bilaterally transplanted with DPSCs in each cerebellar hemisphere (300,000 cells) using a 10-μl Hamilton microsyringe placed at the following coordinates, relative to AP; 11.64; L; ± 2.4 mm; and 5.2 mm DV. In the 3-AP + vehicle group, rats received media as a vehicle. Rats were sacrificed at 30 days post transplantation.

Assessment of motor coordination

A behavioral test was performed 1 day before the injection of 3-AP and at the first, second, third and fourth week after the transplantation of DPSCs. Rats were placed on the accelerating cylinder at speeds increasing from 4 to 40 rpm over a 5-min test session. The test was stopped if the animal fell off the rungs or gripped the device and spun around for two consecutive revolutions without attempting to run. The maximum time that each animal remained on the device was recorded.

Electromyography

Animals were placed under general anesthesia via an intraperitoneal injection of ketamine hydrochloride (60 mg/kg) and xylazine (8 mg/kg). After that, the right hind limb of the animal was shaved and cleaned with a Betadine solution. A 3-cm skin incision was made longitudinally on the posterior aspect of each thigh, from the greater trochanter to the knee. Then, dissection was performed between the gluteus maximus and biceps femoris muscles and the sciatic nerve was exposed, along with the gastrocnemius muscle. With appearance of the sciatic nerve, using forceps cautiously in order to avoid damage to the nerve, it is separated from the surrounding connective tissue so that stimulation electrodes are able to pass under the sciatic nerve. For electrical stimulation, two monopolar subdermal teflon needle electrodes were used, arranged in parallel at a fixed distance of 7 mm from each other. The recording electrodes had an insulating coating, leaving the distal ones uncoated. The sciatic nerve was then stimulated (1 A, 0.2-Hz frequency, 100 s long) and the compound muscle action potential was recorded in the gastrocnemius muscle on the side ipsilateral to the stimulation. The compound muscle action potential parameters analyzed were amplitude and latency. Also during stimulation and recording, a ringer solution was used in order to prevent drying of the nerve.

The rat's cerebellar tissue samples were fixed in 10% formalin for 1 week. Following tissue processing, a proper serial coronal section with a thickness of 10 μm was prepared and was stained with cresyl violet (0.1%). To measure the total volume of white and gray matter using Cavalieri's principle, the following formula was used:

$$V_{(\text{cerebellum})} = \Sigma P \times a/p \times t$$

where t is the distance between the sampled sections. Also, the ΣP was estimated using the point-counting method.

RNA extraction and cDNA synthesis

Total RNA was extracted using High Pure RNA Tissue Kit, according to the manufacturer's instructions (Roche, Basel, Switzerland). Then, 1 μg of total RNA was transcribed to cDNA using murine leukemia virus (MuLV) reverse transcriptase (Fermentas, Lithuania) in the presence of random hexamers and RNase inhibitor.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) analysis was conducted using specific primers for interleukin 1 beta (IL-1β) and tumor necrosis factor (TNF) genes. GAPDH was used as internal control (Table 1). Reactions were performed using SYBR® Premix Ex Taq™ II (Takara Bio Inc.) on a Rotor-Gene™ 6000 real-time PCR machine (Corbett Research, Qiagen,

Table 1 Quantitative real-time PCR primer list

Primer name	Sequence		Annealing	Accession
GAPDH	F	GAACATCATCCCTGCATCCA	60 °C × 25 s	NM_017008.4
	R	GCCAGTGAGCTTCCCGTTCA		
IL-1β	F	AAAGAAGAAGATGGAAAAGC GGTT	60 °C × 25 s	NM_031512.2
	R	GGGAACTGTGCAGACTCAAACTC		
TNF-α	F	ACCACGCTCTTCTGTCTACTG	60 °C × 25 s	NM_012675.3
	R	CTTGGTGGTTTGCTACGAC		

Germany). Initial denaturation was performed at 95 °C for 15 min followed by 40 cycles of denaturation at 95 °C for 5 s, under primer-specific conditions (Table 1) and extension at 60 °C for 20 s. Comparative qPCR quantitation was conducted between candidate groups using REST 2009 (Relative Expression Software Tool, Qiagen).

Immunohistochemistry

Rats were deeply anesthetized by chloral hydrate and perfused transcardially using chilled saline followed by fixative consisting of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Then, the cerebellum was placed in formalin, prepared and placed on slides. The primary antibody was diluted with PBS containing 0.3% Triton X-100 and 1% bovine serum albumin (BSA). Sections were incubated in primary antibody against calbindin (1:500) overnight in 4 °C. Sections were then incubated with the avidin–biotin complex substrate and treated with 0.05% 3,3-diaminobenzidine tetra-hydrochloride and 0.03% hydrogen peroxide in 0.05 M Tris buffer (pH 7.6). After immunohistochemical reaction, sections were mounted, counterstained and observed under a light microscope.

Data analysis

All data are represented as the mean ± SEM. Comparison between groups was made by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The statistical significances were achieved when $P < 0.05$.

Results

Cultured DPSCs express mesenchymal stem cell–specific markers, secrete neurotrophic factors and are differentiable towards osteoblasts and adipocytes under specific differentiation media

When DPSCs are culture-expanded, they demonstrated fibroblast-like cell morphology (Fig. 1a). Flow cytometric analysis revealed that DPSCs were not expressing hematopoietic marker CD45; nonetheless, they displayed the expression of mesenchymal stem cell markers CD90 and CD105 (Fig. 1b). To determine the ability of DPSCs in synthesis of neurotrophic factors VEGF and GDNF, we detected these factors by western blot. Our data showed that DPSCs could express VEGF and GDNF at the protein level (Fig. 1c and Supplementary Fig. 2a, b).

The DPSCs were subjected to an osteogenic medium for a period of 3 weeks that provoked osteogenic lineage formation. This induction was illustrated by precipitation of minerals when the cells were stained with Alizarin Red (Fig. 1d). Additionally, DPSCs were placed in adipogenic medium for 2 weeks, followed by staining with Oil Red, which displayed lipid-containing vacuoles within the cells (Fig. 1e).

Transplantation of DPSCs in rats pre-treated with 3-AP enhanced motor skills and muscle activity

DPSC transplantation was performed 3 days after 3-AP administration, since according to our lab observation, the disease progression was nearly at its highest peak at that day. Similarly, the same transplantation timeline was carried out by Calatrava-Ferreras et al. (2012).

To assess whether the DPSC transplantation in rat cerebellums improved the coordination of movement after injection of 3-AP, the rotarod test was performed. Motor coordination was significantly decreased in the 3-AP-receiving vehicle opposed to the control group. However, after transplantation of DPSCs, motor coordination of the DPSCs + 3-AP group showed a remarkable improvement compared to the 3-AP-receiving vehicle group particularly at the third week ($P < 0.05$) (Fig. 2a). To appraise the efficacy of DPSC implantation on muscle activity, electromyography (EMG) was carried out (Fig. 2b, c and Supplementary Table 1). Latency showed an increase in the 3-AP-receiving vehicle group in comparison with the control group. Following grafting of DPSCs, latency reduced significantly in DPSCs + 3-AP compared with the 3-AP group ($P < 0.05$).

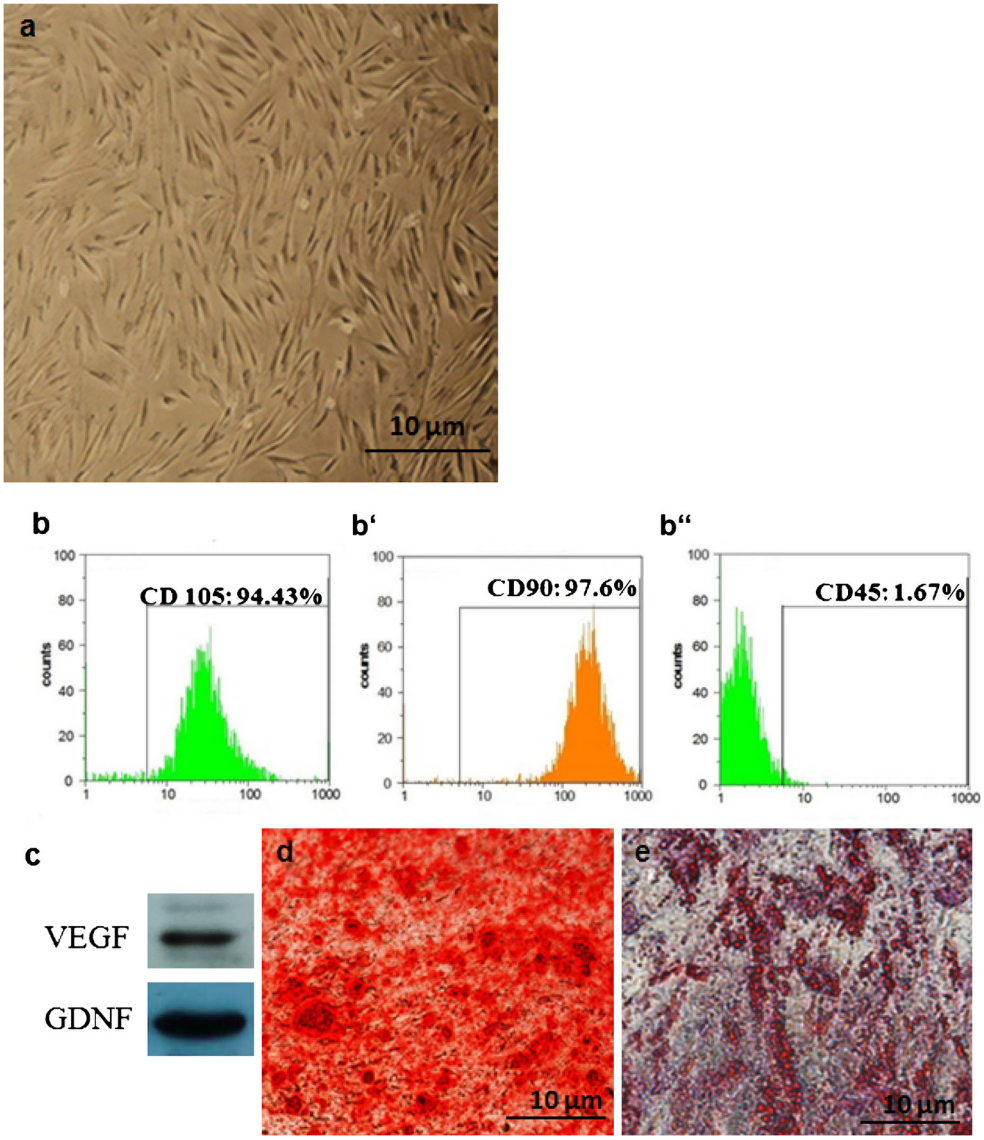


Fig. 1 Culture expansion and in vitro characterization of DPSCs. (a) Fibroblast-like morphology of DPSCs after 2 weeks of isolation. (b, b′, b″) Flow cytometry analysis of DPSCs for the positive expression of MSC markers of CD105 and CD90 while negative for hematopoietic marker CD45. (c) Western blot analysis revealed the synthesis of neurotrophic factors of VEGF and GDNF by DPSCs. Multi-lineage differentiation of DPSCs. (d) Osteogenic cells showing mineral deposits under osteogenic differentiation medium, stained with Alizarin red S. (e) Adipogenic cells displaying lipid droplets under adipogenic differentiation medium, stained with Oil Red O

DPSC implantation precludes the decrease of cerebellar volumes of molecular, granular and white matter layers

Our finding exhibited that the total volume of the molecular layer in the cerebellum was significantly smaller in the 3-AP + vehicle group in comparison to the control group ($P < 0.001$) (Fig. 3a). However, the volume of the molecular layer showed an increase in the DPSCs + 3-AP group in contrast to the 3-AP + vehicle group ($P < 0.05$). Moreover, the total volume of the granular layer in the cerebellum demonstrated a significant drop in the 3-AP + vehicle group compared to the control group ($P < 0.001$) (Fig. 3b). Nevertheless, the volume of the granular layer increased in the DPSCs + 3-AP group compared to the 3-AP + vehicle group ($P < 0.01$). The total volume of the white matter in the cerebellum showed a reduction in the 3-AP + vehicle group compared to the control group ($P < 0.01$) (Fig. 3c). However, the volume of the white matter rose significantly in the DPSCs + 3-AP group compared to the 3-AP + vehicle group ($P < 0.05$).

DPSC implantation thwarted the degeneration of Purkinje cells against 3-AP toxicity

According to our immunohistochemistry results, the mean number of calbindin-positive cells in the cerebellum had shown a noticeable loss in the 3-AP + vehicle group compared to the control group ($P < 0.001$). However, the average number of the Purkinje cells increased in the 3-AP + DPSCs group compared to the 3-AP + vehicle group ($P < 0.05$) (Fig. 4a, b).

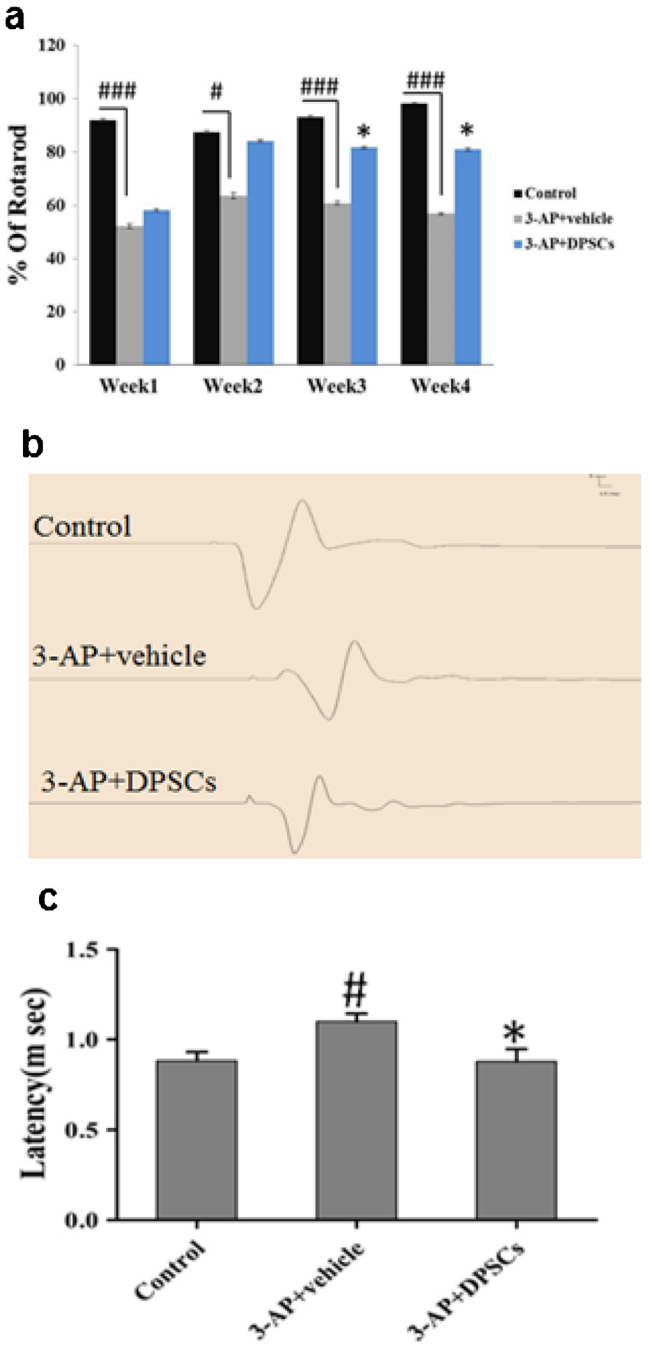


Fig. 2 **a** Transplantation of DPSCs in 3-AP-injected rats improved motor coordination and **b** enhanced muscle EMG activity. The sciatic nerve was stimulated and the compound muscle action potential was recorded in the gastrocnemius muscle. **c** EMG latency was measured in control and 3-AP-injected and DPSC-injected 3-AP. Number sign indicates the difference between the 3-AP-injected group and control, whereas asterisk shows the difference between the DPSC-receiving 3-AP and 3-AP-injected group (*,$^{\#}P < 0.05$; ***,$^{\#\#\#}P < 0.001$). The values are expressed as means (± SEM; $n = 5$)

Transplant of DPSCs diminishes the expression of inflammatory cytokines

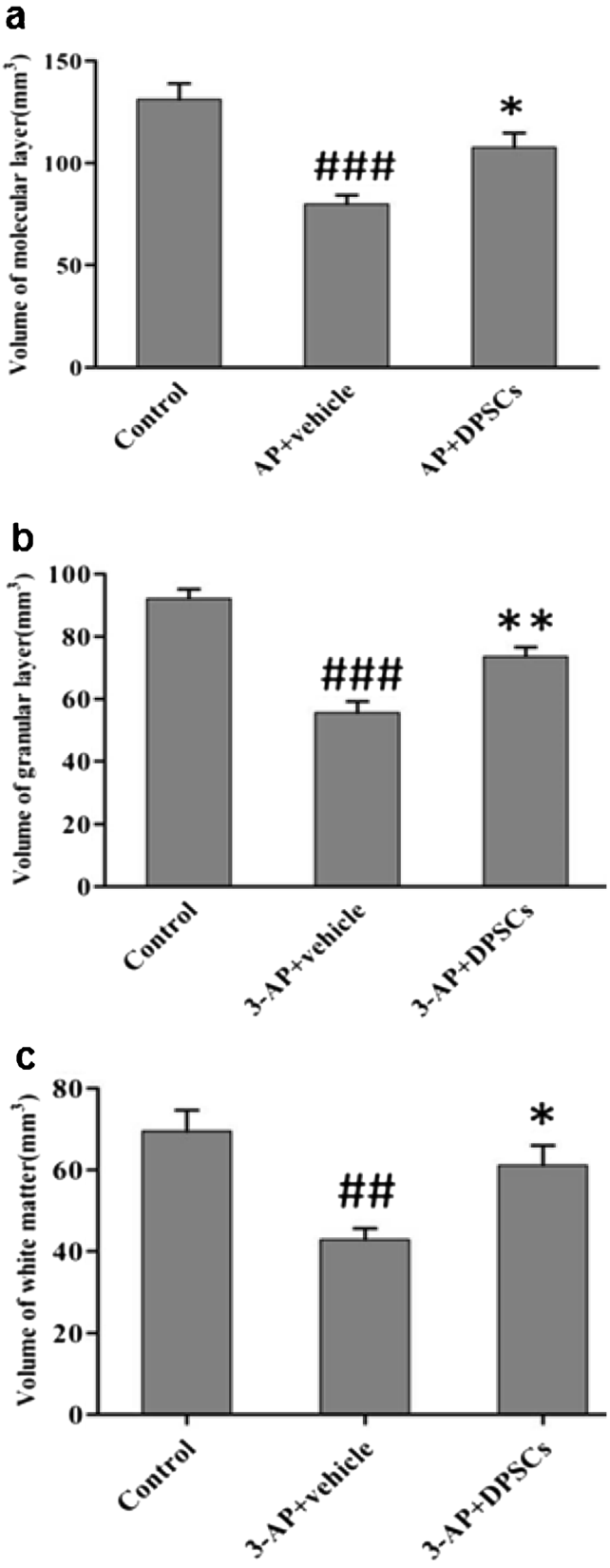
The expressions of genes related to inflammatory cytokines such as *TNF* and *IL-1β* were assessed in the cerebellum 30 days after DPSC transplantation. Quantitative real-time PCR analyses demonstrated that expression of inflammatory cytokines was considerably higher in the 3-AP + vehicle group compared with the control group ($P < 0.001$). However, expression of *TNF* and *IL-1β* decreased in the DPSCs + 3-AP group compared to the 3-AP + vehicle group (Fig. 5).

Discussion

In this study, we demonstrated that transplantation of DPSCs in ataxic rats enhanced motor coordination as well as muscle activity. Also, following cell implantation, the total volume of the molecular and granular layers and volume of the white matter showed a significant rise. Moreover, transplanted DPSCs hindered the denervation of Purkinje cells and reduced the expression of inflammatory cytokines such as *TNF* and *IL-1β*.

Principally, stem cells are immature cells that have a prolonged self-renewability along with the ability to differentiate into multiple cell types. Additionally, transplantation of stem cells has been proposed as a promising remedy for all types of neurodegenerative diseases. Further, well-tailored CRT along with optimized gene therapy procedures could pave the way for clinical translation (Boroujeni and Gardaneh 2018). Owing to the low incidence of adult neural stem cells (NSCs) and related difficulties with harvesting, the use of other stem cell types like MSC with neural potential is highly demanded to achieve neuroregeneration (Tatullo et al. 2015). We previously demonstrated the potential therapeutics of Sertoli cells and umbilical cord matrix stem cells in Huntington's disease (Ahmadi et al. 2018; Ebrahimi et al. 2018).

As shown in past reports, DPSCs possess MSC-like characteristics, including self-renewal capability and multi-lineage differentiation; additionally, it was believed that DPSCs were capable of responding to specific environmental signals in order to generate new cells and/or to select a particular differentiation fate (Da Da Cunha et al. 2013; Gronthos et al. 2002; Miura et al. 2003). DPSCs originated from the cranial neural crest and share neuronal properties, including the production of neurotrophic factors namely nerve growth factor (NGF), BDNF and GDNF (Nosrat et al. 2001). The neuroprotective effects of NGF and BDNF were well established as well



◀ **Fig. 3** Transplantation of DPSCs protected cerebellar volumes of molecular (**a**), granular (**b**) and white matter layers (**c**) against 3-AP neurotoxin. Number sign indicates the difference between the 3-AP-injected group and control, whereas asterisk shows the difference between the DPSC-injected 3-AP and 3-AP-injected group (*,$^{\#}P < 0.05$; **,$^{\#\#}P < 0.01$; ***,$^{\#\#\#}P < 0.001$). The values are expressed as means (± SEM; $n = 5$)

(Heese et al. 2006). Accordingly, we detected the expression of neurotrophic factors such as GDNF and VEGF at the protein level in DPSCs. Moreover, transplantation of DPSCS in spinal cord injury models implied that the surrounding microenvironment chiefly influenced their expression profile. In addition, there were high levels of pro-inflammatory mediators in an injured spinal cord that could activate the oligodendrocyte-specific differentiation (Yamamoto et al. 2014). These reports were consistent with our observations that grafted DPSCs protected cerebellar layers especially Purkinje cells against 3-AP-induced neurotoxicity and alleviated inflammatory response.

DPSCs could be easily expanded and provide large quantities of cells (Yasui et al. 2016). In addition, DPSCs expressed several pluripotent stem cell markers (such as Oct4, Nanog, Sox and Klf4) (Kerkis et al. 2006). These markers possessed potent immunosuppressive and neurogenicity functions (Mead et al. 2014). Due to the invaluable characteristics of DPSCs to differentiate into glial cells and neurons, in addition to their potential to secrete multiple neurotrophic factors (Nosrat et al. 2001; Gronthos et al. 2002; Miura et al. 2003), this investigation was carried out to throw light on the potentiality of DPSCs for CRT. However, further research attempts are highly needed to elucidate whether the DPSCs, besides their neurotrophic and neuroprotective effects, are able to differentiate into entirely functional nerve cells and suitably integrate into a host network. In regard to this, our finding displayed a substantial improvement in motor skills, probably indicating the effectiveness of grafted DPSCs.

Functional recovery of the disrupted human neural system from degenerative diseases had posed a major challenge for CRT strategies (Lindvall and Kokaia 2004). The neuroregeneration/restoration might essentially require synchronicity of processes like neuroprotection, immunomodulation of inflammation, neuroplasticity and neurogenesis. In fact, understanding the underlying molecular and cellular mechanisms might pave the way for any breakthrough in treating neurological disorders (Arthur et al. 2008). Furthermore, Arthur (2008) suggested that transplanted human adult DPSCs had a propensity to differentiate into CNS-type neurons upon exposure to the proper environmental conditions. Likewise, our group previously exhibited the generation of MSC-derived dopaminergic neurons using a specific blend of inducing factors (Boroujeni et al. 2017). However, in the present report, we did not examine the differentiation status of implanted DPSCs, which needs to be explored in future studies.

Transplanted DPSCs altered patterns of axonal migration and may also assist in the homing of endogenous neural stem cells to the site of transplantation (Tatullo et al. 2015).

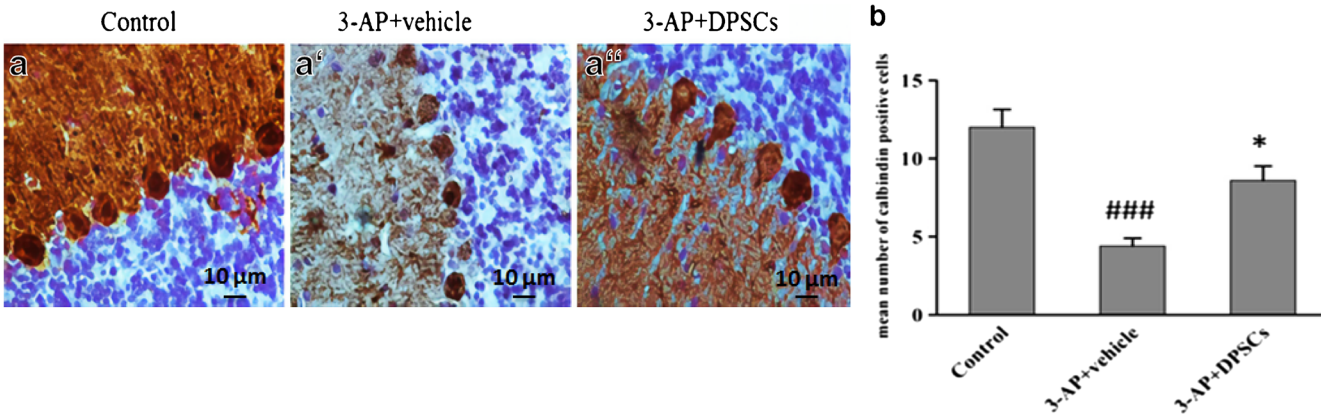


Fig. 4 Immunohistochemistry against calbindin as a neuronal marker. (a, a′, a″) Purkinje cells were immunopositive for anti-calbindin antibody. (b) Mean number of calbindin-positive cells in various groups. The average of calbindin-positive cells was reduced in the 3-AP group in comparison to the control group. Number sign indicates the difference between the 3-AP-injected group and control, whereas asterisk shows the difference between the DPSC-injected 3-AP and 3-AP-injected group (*,$^{\#}P < 0.05$; ***,$^{\#\#\#}P < 0.001$). The values are expressed as means (± SEM; $n = 5$)

Moreover, the grafting of the dental pulp cells (DPCs) disclosed promoted survival of damaged motor neurons in a rat model of spinal cord injury (Nosrat et al. 2001). Similarly, it was revealed that DPSCs have the ability to stimulate long-term regeneration in the injured spinal nerves (Sakai et al. 2012). In another study, DPSCs prevented multiple axon growth suppressors and the apoptosis of neurons, astrocytes and oligodendrocytes in rats with severed spinal cord, resulting in an enhancement in axonal regeneration (de Almeida et al. 2011).

The ability of DPCs to produce and secrete growth factors is of prime importance, since these factors might stimulate the induction of endogenous cell types into those cells required at the place of injury or elicit secretion of other neurotrophic factors from endogenous cells to improve tissue regeneration (Martens et al. 2013). Furthermore, it was shown that transplantation of DPCs into the center of a spinal cord lesion in mice led to preservation of larger areas of white matter and better tissue organization (de Almeida et al. 2011). This was in line with our finding that implanted DPCs reversed the deterioration of cerebellar volume of white matter in ataxic rats. Collectively together, our data imply that DPSC implantation precludes the decline of cerebellar volumes of molecular, granular and white matter layers, since the DPSCs + 3-AP group shows a reduction compared to the control.

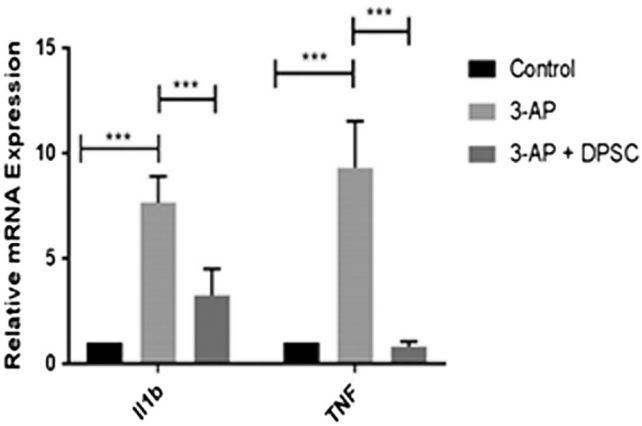


Fig. 5 The gene expression of inflammatory cytokines after transplantation. Transplant of DPSCs declined the gene expression of inflammatory cytokines (*TNF*, *IL-1β*) as shown by quantitative real-time PCR. (***$P < 0.001$). The values are expressed as means (± SEM; $n = 5$)

Conclusion

In summary, we suggest that human DPSCs could be considered as a suitable candidate for CRT with specific focus on CA. This experimental paradigm proved that the transplantation of DPSCs was effective in alleviating 3-AP-induced cerebellar atrophy. Although it might seem impractical to induce fully functional recovery by CRT due to the complexity of the human neural structure and function, any investigation such as the present study prepares the ground to fine-tune the CRT-based approaches.

Acknowledgments This research project was performed at the Cell Biology & Anatomical Sciences Department, Shahid Beheshti University of Medical Sciences.

Funding information This research project was supported by the Vice Chancellor of Research of the Shahid Beheshti University of Medical Sciences.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in the present study involving humans and animals were in accordance with the ethical standards of the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Ahmadi H, Boroujeni ME, Sadeghi Y, Abdollahifar MA, Khodagholi F, Meftahi GH, Hadipour M, Bayat A-H, Shaerzadeh F, Aliaghaei A (2018) Sertoli cells avert neuroinflammation-induced cell death and improve motor function and striatal atrophy in rat model of Huntington disease. J Mol Neurosci 65:17–27

de Almeida FM, Marques SA, Ramalho Bdos S, Rodrigues RF, Cadilhe DV, Furtado D, Kerkis I, Pereira LV, Rehen SK, Martinez AM (2011) Human dental pulp cells: a new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 28:1939–1949

Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 26:1787–1795

Boroujeni ME, Gardaneh M (2017) Umbilical cord: an unlimited source of cells differentiable towards dopaminergic neurons. Neural Regen Res 12:1186–1192

Boroujeni ME, Gardaneh M (2018) The superiority of sucrose cushion centrifugation to ultrafiltration and PEGylation in generating high-titer lentivirus particles and transducing stem cells with enhanced efficiency. Mol Biotechnol 60:1–9

Boroujeni ME, Gardaneh M, Hassan Shahriari M, Aliaghaei A, Hasani S (2017) Synergy between choroid plexus epithelial cell-conditioned medium and knockout serum replacement converts human adipose-derived stem cells to dopamine-secreting neurons. Rejuvenation Res 20:309–319

Brusse E, Maat-Kievit J, Van Swieten J (2007) Diagnosis and management of early-and late-onset cerebellar ataxia. Clin Genet 71:12–24

Butterworth RF, Hamel E, Landreville F, Barbeau A (1978) Cerebellar ataxia produced by 3-acetyl pyridine in rat. Can J Neurol Sci 5(1): 131–133

Calatrava-Ferreras L, Gonzalo-Gobernado R, Herranz AS, Reimers D, Montero Vega T, Jiménez-Escrig A, Richart López LA, Bazán E (2012) Effects of intravenous administration of human umbilical cord blood stemcells in 3-acetylpyridine-lesioned rats. Stem Cells Int 2012:135187. https://doi.org/10.1155/2012/135187

Da Cunha JM, da Costa-Neves A, Kerkis I, da Silva MCP (2013) Pluripotent stem cell transcription factors during human odontogenesis. Cell Tissue Res 353:435–441

Ebrahimi MJ, Aliaghaei A, Boroujeni ME, Khodagholi F, Meftahi G, Abdollahifar MA, Ahmadi H, Danyali S, Daftari M, Sadeghi Y (2018) Human umbilical cord matrix stem cells reverse oxidative stress-induced cell death and ameliorate motor function and striatal atrophy in rat model of Huntington disease. Neurotox Res 34(2): 273–284. https://doi.org/10.1007/s12640-018-9884-4

Fernandez AM, De La Vega AG, Torres-Aleman I (1998) Insulin-like growth factor I restores motor coordination in a rat model of cerebellar ataxia. Proc Natl Acad Sci U S A 95(3):1253–1258

Govindasamy V, Abdullah AN, Ronald VS, Musa S, Ab Aziz ZA, Zain RB, Totey S, Bhonde RR, Abu Kasim NH (2010) Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. J Endod 36:1504–1515

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81:531–535

Heese K, Low JW, Inoue N (2006) Nerve growth factor, neural stem cells and Alzheimer's disease. Neurosignals 15:1–12

Ibarretxe G, Crende O, Aurrekoetxea M, García-Murga V, Etxaniz J, Unda F (2012) Neural crest stem cells from dental tissues: a new hope for dental and neural regeneration. Stem Cells Int 2012:1–12

Janahmadi M, Goudarzi I, Kaffashian MR, Behzadi G, Fathollahi Y, Hajizadeh S (2009) Co-treatment with riluzole, a neuroprotective drug, ameliorates the 3-acetylpyridine-induced neurotoxicity in cerebellar Purkinje neurones of rats: behavioural and electrophysiological evidence. Neurotoxicology 30:393–402

Jiang YY, Cao BB, Wang XQ, Peng YP, Qiu YH (2015) Cerebellar ataxia induced by 3-AP affects immunological function. Neuro Endocrinol Lett 36(3):246–256

Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Massironi SMG, Pereira LV, Caplan AI, Cerruti HF (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184:105–116

Lindvall O, Kokaia Z (2004) Recovery and rehabilitation in stroke. Stem Cells 35:2691–2694

Marmolino D, Manto M (2010) Past, present and future therapeutics for cerebellar ataxias. Curr Neuropharmacol 8:41–61

Marsden J, Harris C (2011) Cerebellar ataxia: pathophysiology and rehabilitation. Clin Rehabil 25(3):195–216

Martens W, Bronckaers A, Politis C, Jacobs R, Lambrichts I (2013) Dental stem cells and their promising role in neural regeneration: an update. Clin Oral Investig 17:1969–1983

Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2014) Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. PLoS One 9:e109305

Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100:5807–5812

Nosrat IV, Widenfalk J, Olson L, Nosrat CA (2001) Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue Motoneurons after spinal cord injury. Dev Biol 238:120–132

Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi H, Kadomatsu K, Ishiguro N, Ueda M (2012) Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 122:80–90

Sierra A, Azcoitia Í, Garcia-Segura LM (2003) Endogenous estrogen formation is neuroprotective in model of cerebellar ataxia. Endocrine 21:43–51

Tatullo M, Marrelli M, Shakesheff KM, White LJ (2015) Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med 9:1205–1216

Wang S, Qu X, Zhao RC (2012) Clinical applications of mesenchymal stem cells. J Hematol Oncol 5(19)

Yamagata M, Yamamoto A, Kako E, Kaneko N, Matsubara K, Sakai K, Sawamoto K, Ueda M (2013) Human dental pulp-derived stem cells protect against hypoxic-ischemic brain injury in neonatal mice. Stroke 44:551–554

Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M (2014) Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res 78:16–20

Yasui T, Mabuchi Y, Toriumi H, Ebine T, Niibe K, Houlihan DD, Morikawa S, Onizawa K, Kawana H, Akazawa C, Suzuki N, Nakagawa T, Okano H, Matsuzaki Y (2016) Purified human dental pulp stem cells promote osteogenic regeneration. J Dent Res 95: 206–214