Stem Cell Reviews and Reports (2019) 15:612–617
https://doi.org/10.1007/s12015-019-09895-2

# Umbilical Cord Cell Therapy Improves Spatial Memory in Aging Rats

Marianne Lehmann[1,2] · Maria F. Zappa-Villar[1,2] · Mariana G. García[3] · Guillermo Mazzolini[3] · Martina Canatelli-Mallat[1,2] · Gustavo R. Morel[1,2] · Paula C. Reggiani[1,2] · Rodolfo G. Goya[1,2]

Published online: 22 May 2019


## Abstract

There is a growing interest in the potential of adult stem cells for implementing regenerative medicine in the brain. We assessed the effect of intracerebroventricular (icv) administration of human umbilical cord perivascular cells (HUCPVCs) on spatial memory of senile (27 mo) female rats, using intact senile counterparts as controls. Approximately one third of the animals were injected in the lateral ventricles with a suspension containing 4.8 X $10^5$ HUCPVC in **8** μl per side. The other third received 4.8 X $10^5$ transgenic HUCPVC overexpressing Insulin-like growth factor-1 (IGF-1) and the last third of the rats received no treatment. Spatial memory performance was evaluated using a modified version of the Barnes maze test. In order to evaluate learning ability as well as spatial memory retention, we assessed the time spent (permanence) by animals in goal sector 1 ($GS_1$) and 3 ($GS_3$) when the escape box was removed. Fluorescence microscopy revealed the prescence of Dil-labeled HUCPVC in coronal sections of treated brains. The HUCPVC were located in close contact with the ependymal cells with only a few labeled cells migrating into the brain parenchyma. After treatment with naïve or IGF-1 transgenic HUCPVC, permanence in $GS_1$ and $GS_3$ increased significantly whereas there were no changes in the intact animals. We conclude that HUCPVC injected icv are effective to improve some components of spatial memory in senile rats. The ready accessibility of HUCPVC constitutes a significant incentive to continue the exploration of their therapeutic potential on neurodegenerative diseases.

**Keywords** Brain aging · Spatial memory · Hippocampus · Umbilical cord · Stem cells

## Introduction

There is a growing interest in the therapeutic potential of adult mesenchymal stem cells (MSCs) in the brain. Although adult tissues are a frequent source of MSCs, they can also be obtained from birth-associated tissues including placenta, amnion, and umbilical cord with the advantage of their availability, avoiding the need for invasive procedures and eliminating other ethical concerns. A number of MSC types can be obtained from the umbilical cord depending on whether they are isolated from the whole umbilical cord (UC-MSCs), Wharton's Jelly (WJ-MSCs), the umbilical cord blood (CB-MSCs) [1], perivascular areas (human umbilical cord perivascular cells or HUCPVCs) [2], In particular, HUCPVCs are advantageous candidates for cell therapy due to their lower donor variability, faster doubling time, and ready availability [3].

Insulin-like growth factor-1 (IGF-1) is a neuroprotective peptide produced by astroglial cells in response to brain insults. We have previously demonstrated that IGF-1 gene therapy in old rats is neuroprotective in different areas of the brain including the hippocampus [4–6]. We therefore reasoned that HUCPVC, genetically modified to express IGF-1 may display a significant restorative effect on spatial memory performance in old rats. In this brief report we describe our results.

## Materials and Methods

**Cells** Human umbilical cord perivascular cells were obtained from healthy donors after written informed consent and protocol was approved by the "Institutional Evaluation Committee" (CIE) from School of Biomedical Sciences,

Marianne Lehmann, Maria F. Zappa-Villar, Paula C. Reggiani and Rodolfo G. Goya contributed equally to this study.

✉ Rodolfo G. Goya
goya@isis.unlp.edu.ar

Paula C. Reggiani
paulareggiani@yahoo.com.ar

[1] INIBIOLP-Pathology B, School of Medicine, National University of La Plata, Calles 60 y 120, 1900 La Plata, Argentina

[2] Department of Histology and of Embryology B, School of Medicine, National University of La Plata, La Plata, Buenos Aires, Argentina

[3] Gene Therapy Laboratory, IIMT, Facultad de Ciencias Biomédicas, CONICET, Universidad Austral, Buenos Aires, Argentina

Austral University (CIE #16–038). The protocol authorizes a number of Hospital researchers to use the human umbilical cords for a number of experimental studies. HUCPVCs were isolated from umbilical cord using a protocol adapted from Sarugaser et al. [2] and were cultured in DMEM low glucose (lg) (Cat# 31600–034; Thermo Fisher Scientific, Buenos Aires, Argentina), as previously described [7].

Cells cultures were grown in Petri dishes to 90% confluence. Suspended MSCs were labeled with 1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine perchlorate (Dil, Sigma Chem Co. cat# D8417) fluorescent dye (stock solution: 0.25 μg of Dil per microliter of dimethylsulfoxide). Briefly, trypsinized MSCs were suspended in phosphate buffered saline (PBS, $10^6$ cells/ml) in the presence of Dil at a final concentration of 1 μg/ml and incubated for 5 min at 37 °C followed by 15 min at 4 °C and finally washed 3 times with PBS.

**Cell Transduction** HUCPVCs were seeded at 70% of confluence in complete medium. Medium was then removed, and cells were transduced with either an adenoviral vector expressing the cDNA for rat IGF-1 (RAd-IGF-1), or an adenoviral vector (RAd-GFP) expressing the reporter gene encoding humanized green fluorescent protein (GFP) in both cases at a multiplicity of infection (MOI) of 30 in DMEM lg and 2% fetal bovine serum (FBS; Natocor, Inc. Cordoba, Argentina) in half of total volume for 2 h. The RAd-GFP-transduced cells were incubated for 5 min with (1 μg/ml) DAPI (4,6-Diamidino-2-phenylindole, dihydrochlorid; Sigma Chem Co. cat# 10236276001), only.

Subsequently, medium was completed with 10% FBS in DMEM lg. Three days later cells were processed and injected as described below.

**RAd-GFP and RAd-IGF1** were constructed employing the adenovector construction kit AdMax from Microbix Biosystems, Mississauga, Canada.

**Animals** Senescent (27 mo.) female Sprague-Dawley (SD) rats were used. The animals were housed in a temperature-controlled room (22 ± 2 °C) on a 12:12 h light/dark cycle. Food and water were available ad libitum. All experiments with animals were performed in accordance with the Animal Welfare Guidelines of NIH (INIBIOLP's Animal Welfare Assurance No A5647–01) and institutional IACUC-approved protocol P05-02-2015.

Rats were grouped, as follows: **Group Intact**, consisted of 6 senile rats that received no treatment. **Group HUCPVC**, consisted of 8 senile rats that were stereotaxically injected with HUCPVC only and **Group HUCPVC + IGF-I**, consisted of 9 senile rats that were stereotaxically injected with HUCPVC transduced with RAd-IGF-1.

**Experimental Design** Nine days before HUCPVC injection (experimental day −9) the pre-treatment Barnes test began. On experimental day 0, experimental rats were icv injected with HUCPVCs as described below. From experimental day 21 to 26 all rats were submitted to the post-treatment Barnes test **(**Fig. 1a). On experimental day 27 all rats were sacrificed as described below.

## Surgical Procedures

**Stereotaxic Injections** Rats were anesthetized with ketamine hydrochloride (40 mg/kg; ip) plus xylazine (8 mg/kg; im) and placed in a stereotaxic apparatus. In order to access the lateral ventricles (LV), the tip of a 26G needle fitted to a 50 μl syringe was brought to the following coordinates relative to the bregma: −0.8 mm anteroposterior, 4.1 mm dorsoventral and ± 1.5 mm mediolateral [8]**.** The animals were injected bilaterally with 8 μl per side of a suspension containing 4.8 X $10^5$ HUCPVC.

## Spatial Memory Assessment

The modified Barnes maze protocol used in this study was based on a previously reported procedure [9]. It consists of an elevated (108 cm above the floor) black acrylic circular platform, 122 cm in diameter, containing 20 holes around the periphery **(**Fig. 2 **A inset).** The holes are uniform in diameter (10 cm) and appearance, but only one hole is connected to a black escape box. The escape box is 38.7 cm long × 12.1 cm wide × 14.2 cm in depth and it is removable. A white cylindrical starting chamber (an opaque, 35 cm diameter and 20 cm high, open-ended chamber) is used to place the rats on the platform. Four proximal visual cues are located in the test room, 50 cm from the platform. The escape hole was numbered as hole 0 for graphical normalized representation purposes, the remaining holes being numbered 1 to 10 clockwise, and − 1 to −9 counterclockwise **(**Fig. 2a **inset)**. Hole 0 was held in a fixed position, relative to the cues to avoid randomization of the relative position of the escape box. During the tests the platform was rotated daily. A 90-dB white-noise generator and a white-light 500 W bulb provided the escape stimuli from the platform.

We used an abbreviated protocol based on three days of acquisition trials (AT), followed by two probe trials (PT), 1 and 5 days after training (PT1 and PT2), to assess recent spatial memory retention. An AT consists of placing a rat, randomly oriented, in the starting chamber for 30s. The chamber is then raised, the aversive stimuli are switched on and the rat is allowed to freely explore the maze for 120 s. A probe trial is defined as a trial where the escape box has been removed, its purpose being to assess the latency to explore the empty escape hole and the error frequency. After the starting chamber is raised, the rat is given 120 s to explore and the number of explorations per hole is recorded. In order to

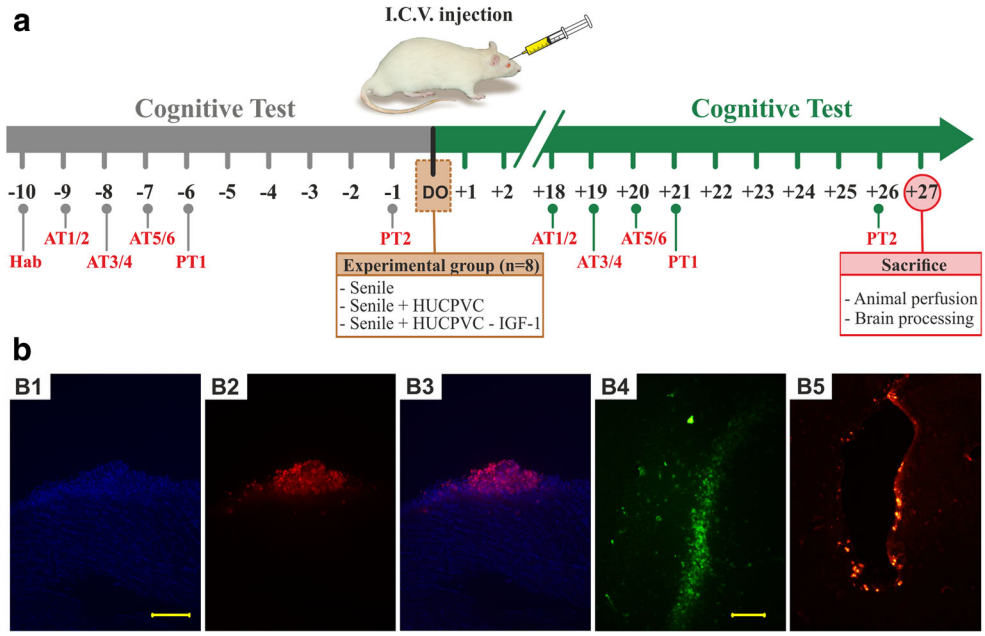


**Fig. 1 Experimental Design and HUCPVC cell visualization in vitro and in vivo. Panel A**, illustrates the experimental design of the study. All animals were submitted to 2 daily AT from experimental day −9 to −6 and to two PT on experimental day −6 and − 1 and at experimental day 21 and 26. The naïve and transduced cells were stereotaxically injected in the lateral ventricles of of experimental rats on experimental day 0. From experimental day 18 to 20 all rats received a second (post treatment) series of AT and two additional PTs on experimental day 21 and 26. On experimental day 27 rats were sacrificed, perfused with fixative and the brains removed for morphological analysis. Hab, habituation; AT, acquisition trial; PT, probe trial. **Panel B- Representative images of HUCPVC in the ventricular spaces 5 days post injection. B1** DAPI-labeled region of the LV. **B2-** Dil-labeled HUCPVC. **B3-**Merge of B1 and B2. **B4-** RAd-GFP-transduced HUCPVC in the LV, fluorescence microscopy. **B5-**RAd-IGF1-transduced HUCPVC in the LV, fluorescence microscopy. Abbreviation: LV, lateral ventricle. B1. B2 and B3, scale bar, 300 μm. B4 and B5, scale bar, 200 μm

eliminate olfactive clues from the maze and the boxes, the surfaces are cleaned with 10% ethylic alcohol solution, after each trial. On the day before AT, animals were habituated to the starting chamber and escape box by placing them inside each one for 60 s in the starting chamber and 120 s in the escape box

Behavioral performance was recorded using a computer-linked video camera mounted 110 cm over the platform. The video-recorded performances of the subjects were measured using the Kinovea v0.7.6 (http://www.kinovea.org) software. The behavioral parameters assessed were as follows.

(a). Hole exploration frequency in a goal sector (GS): the sum of the number of explorations for holes −1, 0, and 1 divided by 3 **($GS_3$)** or the number of explorations of hole 0 **($GS_1$),** during a PT.
(b). Permanence in GS1 and GS3: the time (in s) that a rat spends in the GS corresponding to hole 0 or holes -1, 0 and +1, during a PT1 and PT2.

## Brain Processing

Animals were placed under deep anesthesia and perfused with phosphate buffered para-formaldehyde 4%, (pH 7.4) fixative. The brains were rapidly removed and stored in para-formaldehyde 4%, (pH 7.4) overnight (4 °C). Finally, brains were maintained in cryopreservative solution at −20 °C until use.

## Statistical Analysis

Data were analyzed with the Sigma Plot v. 11 software (San Jose, CA). Comparisons between groups were made using one-way ANOVA for the probe trials. Data are presented as mean ± SEM. Tukey's post-hoc tests was used where appropriate. Criteria for significant differences were set at 95% probability level.

# Results

## Time-Course of HUCPVC in the Cerebral Ventricles

Dil-fluorescence in coronal sections of HUCPVC-injected brains revealed that five days after injection, Dil-labeled cells were located in close contact with the ependymal cells (Fig. 1b). The number of labeled cells decreased progressively from Experimental day 5 on. On the day of sacrifice labeled cells were still observable close to the ependymal cells (data not shown).

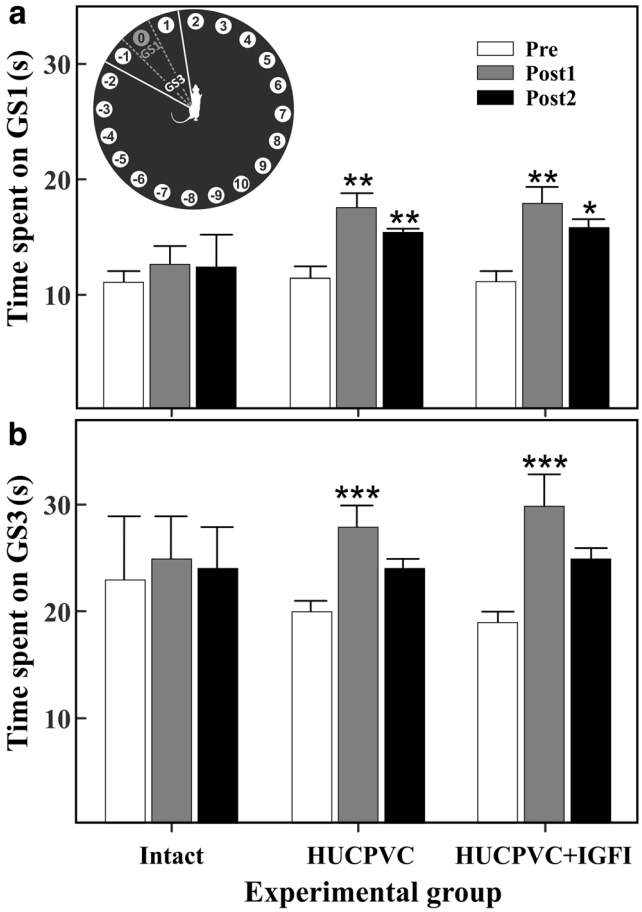


**Fig. 2 Effect of MSCs treatment in old rats on $GS_1$ and $GS_3$ sector permanence-** Sector permanence (expressed as the time spent in the corresponding sector) in the Senile (intact) group showed no significant changes 27 days after the initial tests (equivalent to pre versus post treatment period in the other groups) in either $GS_1$ or $GS_3$. In contrast, in both, the HUCPVC and the HUCPVC+IGF-1 experimental groups post-treatment sector permanence increased significantly for PT1 and PT2. In all cases, comparisons were made versus the corresponding pre treatment counterparts. **Inset-** Barnes maze platform showing GS1 and GS3. *, $P < 0.05$: **, $P < 0.01$, **, $P < 0.001$, $N = 6$, 8 and 9 for Senile control, HUCPVC and HUCPVC+IGF-1, respectively in both PT1 and PT2

### Cognitive Changes

**Hole Exploration** The hole exploration frequency of senile rats before and after treatment was assessed and compared in different goal and nongoal sectors. No significant differences were detected (data not shown).

### Time Spent in $GS_1$ and $GS_3$

The time spent by rats from different experimental groups in $GS_1$ and $GS_3$ before and after treatment at PT1 and PT2 was compared. In the intact senile rats there was no significant difference between pre and post-treatment groups in $GS_1$ **(**Fig. 2a**).** In contrast, in the HUCPVC and HUCPVC+IGF-1 groups, the time spent in $GS_1$ was significantly longer at PT1 and PT2 than at pre-treatment. For $GS_3$, in the intact senile rats there was no significant difference between pre- and post-treatment whereas in the HUCPVC and HUCPVC+IGF-1 groups the time spent in $GS_3$ was significantly longer at PT1, but not PT2, than at pre- treatment **(**Fig. 2b**).**

## Discussion

Adult stem cells constitute a promising therapeutic tool for the treatment of human neurodegenerative processes. Thus, a number of irreversible neurodegenerative diseases, such as Alzheimer's Disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD), stem cell therapy constitutes a promising new avenue of research. Although a number of pre-clinical studies have been launched, quite a few clinical studies have been performed at present. Evidence suggests that autologous bone-marrow derived mesenchymal stem cells are safe and effective when transplanted unilaterally in PD patients. Interestingly, no adverse effects of these stem cells were detected, a fact that paved the way for future studies [10]**.** Human umbilical cord blood derived MSC were transplanted in AD patients at two different doses and end-point analysis was measured by ADAS-cog scoring, PET imaging, and the measurement of Aβ and tau levels in CSF [11]. In China, a research group recruited AD patients in phase I/II trial with thirty probable AD participants, using a similar study design as the one outlined above, where patients are intravenously administered with 20 X10 [6] human UCB-MSCs [12]. Medipost Co Ltd. started a double blinded, placebo controlled, phase I/IIa trial. Patients with mild to moderate AD will be subjected to repeated intraventricular administrations of UCB-MSCs and will be evaluated 24 weeks after the first transplantation [13]**.**

Concerning animal studies, in 2015 we demonstrated that lifelong intravenous (iv) injection of hMSCs begun in a rat at 6 months of age, markedly prolonged lifespan (44 months versus the typical 36 months of standard laboratory rats) [14]**.** Our findings are in line with a previous study where lifelong iv injection of amniotic membrane-derived hMSCs (AM-MSCs) or adipose tissue-derived MSC (AD-MSC) to 10-month-old male F344 had a similar life-extending effect. The AM-MSC and AD-MSC improved cognitive and physical functions of naturally aging rats, extending life span by 23 and 31%, respectively [15]. This and other [16] evidence prompted us to undertake the present study. We reasoned that icv administration of HUCPVC to aged rats could be able to restore their deficient cognitive performance.

The time-course results reveal a slowly declining number of HUCPVC in the cerebroventricular space until the end of the experiment. A rather modest number of Dil(+) cells were observed in the brain parenchyma at any time. The initial assumption concerning the mechanisms by which MSCs

improve central nervous system (CNS) injury, was that they migrate to the injured tissues and transdifferentiate to replace injured neural cells [17, 18]. However, subsequent studies showed that transplanted MSCs may exert their therapeutic effect without evidence of engraftment [19, 20], thus indicating that their regenerative and differentiating abilities do not play a role in limiting tissue destruction or enhancing tissue repair. Instead, it seems that inflammatory signals, induce MSCs to secrete a variety of bioactive molecules, such as anti-inflammatory molecules and trophic factors able to modulate the host microenvironment [21], which seems to be the main mechanism responsible for their therapeutic effects when they are administered icv or iv [22]. Therefore, the putative neurogenic factors released by our HUCPVC into the cerebroventricular space should have reached hippocampal cells thus inducing a moderate improvement in function.

In laboratory rodents, spatial memory has been extensively evaluated using the Morris Water Maze (MWM). However, a potential disadvantage of the MWM in age-related studies is that it requires a substantial degree of physical fitness. Thus, we chose the less physically demanding Barnes maze for evaluating spatial memory in aging rats. In both tests, rats learn to use spatial cues to guide them to a hidden platform or tunnel. While, the MWM involves immersion in water, a stimulus that induces considerable corticosterone secretion and corticotropin release [23], in the Barnes maze rodents are placed on an circular platform for them to walk in search of the escape hole.

The MWM probe trial, which specifically measures the spatial component of target-finding, has no counterpart in the Barnes maze. However, in more recent times, removal of the escape box was used to measure spatial memory extinction [24]. By removing the escape box at the end of the training period the PT was introduced in our variant of the test, which enabled us to assess memory-dependent variables as well as recent (PT1) and longer-term (PT2) retention of spatial memory, whose levels are proportional to the inverse of spatial memory extinction. In this study we found that the permanence in $GS_1$ and $GS_3$ during PT1 and PT2 is a sensitive parameter to assess the effect of HUCPVC-treatment on spatial memory. Hole exploration frequency was less sensitive to the treatement.

Concerning the effect of HUCPVC on the permanence in GS1 and GS3, our findings are in line with documented evidence of cognitive improvement in rodent models of neurodegenerative diseases. Thus, it has been reported that in an AD mouse model, intra-hippocampal administration of human umbilical cord blood-derived mesenchymal stem cells (hUC-MSCs) significantly rescued cognitive performance as determined by the MWM test [25]. In another study, adult human MSCs were administered ($10^5$ cells/bilaterally) into the lateral ventricle of Flinders sensitive line (FSL) rats, an animal model for depression. When 12 days later, animals were assessed by the forced swim test and the dominant–submissive relationship (DSR) paradigm, results revealed that MSCs-transplanted FSL rats had a clear improvement in their behavioral performance in both tests [26].

It has been previously shown that transgenic mouse bone marrow-derived mesenchymal stromal cells overexpressing IGF-1 seem to possess an anti-fibrogenic effect on experimental hepatic fibrosis induced in mice by chronic thioacetamide application or bile duct ligation [27]. Our results suggest that overexpression of IGF-1 in our HUCPVC does not enhance the restorative activity of HUCPCV per se on the permanence performance of old rats in $GS_1$ and $GS_3$. It could be speculated that at hippocampal level, the restorative action of HUCPVC-derived soluble factors exert a maximal restorative action on neurons and/or glia, thus preventing further improvement by transgenic IGF-1 action.

**Acknowledgements** The authors are indebted to Ms. Natalia Scelsio for technical work, to Mr. Mario R. Ramos for design of the figures and to Ms. Yolanda E. Sosa for editorial assistance. MGG, GM, GRM, PCR and RGG are career researchers of the Argentine Research Council (CONICET). ML, FZ and MCM are recipients of CONICET doctoral fellowships.

**Funding** This study was supported by grant #PICT15–0817 from the Argentine Agency for the Promotion of Science and Technology and grant MRCF 10–10-17 from the Medical Research Charitable Foundation and the Society for Experimental Gerontological Research, New Zealand to RGG.

## Compliance with Ethical Standards

**Conflict of Interest** There are no conflicts of interest concerning any of the authors.

## References

1. Hass, R., Kasper, C., Böhm, S., & Jacobs, R. (2011). Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Communication Signaling., 9*, 12.
2. Sarugaser, R., Lickorish, D., Baksh, D., Hosseini, M. M., & Davies, J. E. (2005). Human umbilical cord perivascular (HUCPV) cells: A source of mesenchymal progenitors. *Stem Cells, 23*, 220–229.
3. Baksh, D., Yao, R., & Tuan, R. S. (2007). Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells, 25*, 1384–1392.
4. Hereñú, C. B., Cristina, C., Rimoldi, O. J., et al. (2007). Restorative effect of insulin-like growth factor-I gene therapy in the hypothalamus of senile rats with dopaminergic dysfunction. *Gene Therapy., 14*, 237–245.
5. Pardo, J., Uriarte, M., Console, G. M., et al. (2016). Insulin-like growth factor-I gene therapy increases hippocampal neurogenesis, astrocyte branching and improves spatial memory in aging rats. *European Journal of Neuroscience, 44*, 2120–2128.
6. Pardo, J., Abba, M., Lacunza, E., et al. (2017). Identification of a conserved gene signature associated with an exacerbated inflammatory environment in the hippocampus of aging rats. *Hippocampus, 27*, 435–449.

7. Bayo J., Fiore, E, Aquino, J B, et al. (2014) Human umbilical cord perivascular cells exhibited enhanced migration capacity towards hepatocellular carcinoma in comparison with bone marrow mesenchymal stromal cells: A role for autocrine motility factor receptor. *Biomed. Res. Int.* https://doi.org/10.1155/2014/837420.
8. Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic Press.
9. Morel, G. R., Andersen, T., Pardo, J., et al. (2015). Cognitive impairment and morphological changes in the dorsal hippocampus of very old female rats. *Neuroscience, 303*, 189–199.
10. Venkataramana, N. K., Kumar, S. K., Balaraju, S., et al. (2010). Openlabeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. *Translational Research, 155*, 62–70.
11. The Safety and The Efficacy Evaluation of NEUROSTEM®-AD in Patients With Alzheimer's Disease. ClinicalTrialsgov Identifier: NCT01297218. 2014. http://clinicaltrials.gov/ct2/show/NCT01297218.
12. Safety and efficiency of umbilical cord-derived mesenchymal stem cells (UC-MSC) in patients with Alzheimer's disease (SEMAD). Clinical Trialsgov Identifier: NCT01547689. May. 2014 https://clinicaltrials.gov/ct2/show/NCT01547689
13. Safety and exploratory efficacy study of NEUROSTEM® versus placebo in patients with Alzheimer's disease. Clinicaltrialsgov identifier: nct02054208. Feb. 2014 https://clinicaltrials.gov/ct2/show/NCT02054208
14. Mansilla, E., Roque, G., Sosa, Y. E., Tarditti, A., & Goya, R. G. (2016). A rat treated with mesenchymal stem cells lives to 44 months of age. *Rejuvenation Research., 19*, 318–321.
15. Kim, D., Kyung, J., Park, D., et al. (2015). Health span-extending activity of human amniotic membrane- and adipose tissue-derived stem cells in F344 rats. *Stem Cells Translational Medicine, 4*, 1144–1154.
16. Shen, J., Tsai, Y., DiMarco, N., Sun, X., & Tang, L. (2011). Transplantation of mesenchymal stem cells from young donors delays aging in mice. *Science Reports, 1*, 67.
17. Kopen, G. C., Prockop, D. J., & Phinney, D. G. (1999). Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proceedings of the national Academy of Sciences USA, 96*, 10711–10716.
18. Qu, C., Mahmood, A., Lu, D., Goussev, A., Xiong, Y., & Chopp, M. (2008). Treatment of traumatic brain injury in mice with marrow stromal cells. *Brain Research, 1208*, 234–239.
19. Janowski, M., Wagner, D. C., & Boltze, J. (2015). Stem cell-based tissue replacement after stroke: Factual necessity or notorious fiction? *Stroke, 46*(*8*), 2354–2363.
20. Wang, N., Li, Q., Zhang, L., et al. (2012). Mesenchymal stem cells attenuate peritoneal injury through secretion of TSG-6. *PLoS One, 7*, e43768.
21. Caplan, A. I., & Dennis, J. E. M. (2006). Mesenchymal stem cells as trophic mediators. *Journal of Cellular Biochemistry, 98*, 1076–1084.
22. da Silva Meirelles, L. S., Fontes, A. M., Covas, D. T., & Caplan, A. I. (2009). Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Reviews., 20*, 419–427.
23. Sternberg, E. M., Glowa, J. R., Smith, M. A., et al. (1992). Corticotropin releasing hormone related behavioral and neuroendocrine responses to stress in Lewis and Fischer rats. *Brain Research., 570*, 54–60.
24. Vargas-Lopez, V., Lamprea, M. R., & Munera, A. (2011). Characterizing spatial extinction in an abbreviated version of the Barnes maze. *Behavioural Processes, 86*, 30–38.
25. Lee, H. J., Lee, J. K., Lee, H., et al. (2010). The therapeutic potential of human umbilical cord blood-derived mesenchymal stem cells in Alzheimer's disease. *Neuroscience Letters, 481*, 30–35.
26. Tfilin, M., Sudai, E., Merenlender, I., Gispan, G., Yadid, G., & Turgeman, G. (2010). Mesenchymal stem cells increase hippocampal neurogenesis and counteract depressive-like behavior. *Molecular Psychiatry, 5*, 1164–1175.
27. Fiore, E. J., Bayo, J. M., Garcia, M. G., et al. (2014). Mesenchymal stromal cells engineered to produce IGF-I by recombinant adenovirus ameliorate liver fibrosis in mice. *Stem Cells Development, 24*, 791–801.