PLOS ONE

RESEARCH ARTICLE

Comparative study of the neural differentiation capacity of mesenchymal stromal cells from different tissue sources: An approach for their use in neural regeneration therapies

Daniela N. Urrutia[1], Pablo Caviedes[2,3], Rodrigo Mardones[1,4], José J. Minguell[1], Ana Maria Vega-Letter[5], Claudio M. Jofre[1]*

1 Regenerative Cell Therapy Center, Clinica Las Condes, Santiago, Chile, **2** Program of Molecular & Clinical Pharmacology, ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile, **3** Centro de Biotecnología y Bioingeniería (CeBiB), Departamento de Ingeniería Química, Biotecnología y Materiales, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile, **4** Orthopedic Department, Clinica Las Condes, Santiago, Chile, **5** Program of Traslational Immunology ICIM, Faculty of Medicine, Clinica Alemana Universidad del Desarrollo, Santiago, Chile

* claudio.jofre@bioscell.cl

OPEN ACCESS

Citation: Urrutia DN, Caviedes P, Mardones R, Minguell JJ, Vega-Letter AM, Jofre CM (2019) Comparative study of the neural differentiation capacity of mesenchymal stromal cells from different tissue sources: An approach for their use in neural regeneration therapies. PLoS ONE 14(3): e0213032. https://doi.org/10.1371/journal.pone.0213032

Editor: Gianpaolo Papaccio, Università degli Studi della Campania, ITALY

Received: November 20, 2018

Accepted: February 13, 2019

Published: March 11, 2019

Data Availability Statement: All relevant data are within the manuscript, Supporting Information files, and at www.ebi.ac.uk/biostudies/studies/S-BSST236 with the accession number S-BSST236 (www.ebi.ac.uk/biostudies/studies/S-BSST236).

Funding: This study was supported by RM-13IEAT-20943 from innova Corfo, https://www.corfo.cl/, CJ, and PI2013-DA001 from Academic

Abstract

Mesenchymal stem cells (MSCs) can trans/differentiate to neural precursors and/or mature neurons and promote neuroprotection and neurogenesis. The above could greatly benefit neurodegenerative disorders as well as in the treatment of post-traumatic and hereditary diseases of the central nervous system (CNS). In order to attain an ideal source of adult MSCs for the treatment of CNS diseases, adipose tissue, bone marrow, skin and umbilical cord derived MSCs were isolated and studied to explore differences with regard to neural differentiation capacity. In this study, we demonstrated that MSCs from several tissues can differentiate into neuron-like cells and differentially express progenitors and mature neural markers. Adipose tissue MSCs exhibited significantly higher expression of neural markers and had a faster proliferation rate. Our results suggest that adipose tissue MSCs are the best candidates for the use in neurological diseases.

Introduction

Mesenchymal stem cells (MSCs) are a class of adult stem cells, which undergo self-renewal and exhibit pluripotency [1]. In addition, MSCs have immunomodulatory properties, produce trophic factors for tissue repair/regeneration [2, 3], and differentiate into various cell lineages, including neurons and glial cells [4, 5].

MSCs were originally identified in the bone marrow [6], they have also been found in other locations such umbilical cord tissue [7], umbilical cord blood [8] adipose tissue [9] skin [10] teeth [11, 12] and pancreas [13]. Among all these tissues, adipose, skin and umbilical cord are

Direction of Clinica Las Condes, www.clinicalascondes.cl.

Competing interests: The authors have declared that no competing interests exist.

attractive choices to obtain cells due to the relatively easy access to samples in clinical settings [10, 14–16].

Accordingly, MSCs properties have laid a solid foundation for their clinical application in the field of regenerative medicine [17, 18]. Furthermore, a precise characterization of MSCs derived from different tissues sources represent an essential requirement for the development of MSC-based therapies to repair and/or regenerate damaged tissues.

In the specific case of the central nervous system, nervous tissues show the most limited regeneration and recovery capabilities after injury. In humans, neurogenesis is restricted to the dentate gyrus of the hippocampus and, despite the existence of endogenous neural stem cells, their capacity is not enough to induce full repair and regeneration [19]. These facts account for the devastating nature of many neurological diseases where recovery is incomplete and major disability often results. Accordingly, the search for new sources of stem cells with potential to differentiate into a neural phenotype represents a central issue for the treatment of neurodegenerative conditions, post-traumatic and/or hereditary diseases.

In this regard, the promising results of animal and human studies using MSCs from several tissue sources [20–28], have presented the possibility of using these cells for neural repair. Nevertheless, *in vitro* studies using MSCs isolated from bone marrow and adipose tissue have shown variability in their ability to differentiate toward a particular mature neural lineage [29, 30], to generate functional neurons [31], as well as to support neural regeneration after transplantation [32]. Since these variations may result in heterogeneous clinical outcomes, there is a need to establish a relevant MSC source for neurological repair and regeneration.

In light of the above, the aim of this study was to evaluate the neural differentiation capacity of *ex vivo* expanded MSCs isolated from several human tissues, including adipose, bone marrow, skin and umbilical cord. The data from the studies described herein may be valuable for selecting the proper tissue source of MSC to be used therapeutically in neural regenerative therapies.

Materials and methods

1. Collection and isolation of MSCs from the different tissue sources

This study was performed at the Regenerative Cell Therapy Center, Clinica Las Condes, Santiago, Chile. Procedures carried out in this study complied with regulations and were approved by the Research and Ethics Committees of Clinica Las Condes. All donors and/or their parents gave written informed consent for the use of the requested tissue. Average age of donors was 28 ± 5 years (with the obvious exception of umbilical cord), gender ratio (male/female) was 7:2 (Table 1). No donors used concomitant drugs.

Mesenchymal Stem Cells (MSCs) were obtained from Adipose Tissue (AT), Bone Marrow (BM), Skin, and Umbilical Cord (UC) (Table 1). For isolation of MSCs, the respective tissues were processed according to indications [33] for Bone Marrow-derived MSC (BM-MSC) and

Table 1. Tissue sources characteristics of MSCs utilized in this study.

Tissue Source	Donor patient age average	Donor patient gender n = 3	Subculture passage(#) used
Bone Marrow	27	All male	3–5
Adipose	28	2 female 1 male	3–5
Skin	29	All male	3–4
Umbilical Cord	new born	-	3–5

Tissue sources characteristics of MSCs utilized in this study. Donor patient age average and gender were n = 3.

https://doi.org/10.1371/journal.pone.0213032.t001

Adipose Tissue-derived MSC (AT-MSC), described in [34] for Umbilical Cord-derived MSC (UC-MSC), and indicated in [35] for Skin-derived MSC (SD-MSC). BM aspirates (n = 3) were obtained from the iliac crest. AT samples were obtained during abdominal plastic surgeries (n = 3). Ten-centimeter-long UC were collected and donated from consenting patients delivering full-term infants by caesarian section (n = 3). Pieces of skin tissue from arms (n = 3) were carefully dissected free of other tissue and cut into 2–3 mm^3 pieces.

2. Culture and *ex vivo* expansion of MSCs obtained from the different tissues sources

MSCs-derived from the above-indicated tissue sources were cultured under the same culture conditions: growth medium, consisting in Minimum Essential Medium alpha modification (α - MEM, Gibco-Invitrogen, USA) supplemented with 10% Fetal Bovine Serum (FBS, Corning Cell Gro), 1X penicillin–streptomycin (Pen-strep, Biological Industries). As soon as a culture reached confluence, cells were expanded. In all studies, resulting MSCs at passage 3 to 5 were used.

3. Immunophenotyping

Cultures of Isolated MSCs obtained from the different origins were labeled with the following anti-human antibodies: CD11b-AF488, CD29-PE, CD73-PE, CD90-FITC, CD105-PE (BD Bioscience), CD34-PE, CD19-PE, CD45-FITC (Beckman C), and HLADR-PE from Invitrogen. Mouse isotype antibodies served as respective controls (Invitrogen). Labelled cells were analyzed using a FACS-Vantage-SE flow cytometry system running CellQuest software (BD). The fluorescence signals were collected using logarithmic amplification.

4. Population doubling time

To examine MSCs Population Doubling Time (PDT), cells at passage 3 were seeded at a density of $5X10^3$ cm^2 and PDT calculated by using an algorithm available online (http://doubling-time.com) [36].

5. Adipogenic, chondrogenic and osteogenic differentiation

To assess adipogenic, chondrogenic and osteogenic differentiation, cells were cultured in basal medium until 70–80% confluence and then changed to every induction medium and stained [35, 37]. Images were obtained with microscope NIKON ECLIPSE Ti-s.

6. Neural induction

The induction protocol was adapted from [38–40]. Briefly, MSCs neural specification (step 1) was induced by culturing cells in α MEM supplemented with: 0,25X B27, 1X N2, 20 ng/mL EGF and 20 ng/mL FGF basic for 5 days. At the end of the neural specification treatment, MSCs were washed with PBS, and then neuronal commitment (step 2) was induced by exposing the cells to α MEM supplemented with 0,25X B27, 100 ng/mL Sonic HedgeHog, 2,5 μM Retinoic Acid and 1 mM AMPc during the next 10 days. Finally, we induced neuronal differentiation (step 3) adding 30 ng/mL BDNF during the final 3 days. One non-induced culture dish was also analyzed in every experiment as negative control. Neural Stem Cells (NSC) from StemPro were used as positive control and differentiated as indicated by the supplier. The cells were monitored continually after neuronal induction. The area in pixels were measured by ImageJ with "measure" function, and neurite quantification with NeuronJ pluggin.

7. Reverse transcription polymerase chain reaction RT–PCR

To detect gene expression indicative of MSCs neural differentiation, mRNA was harvested using trizol (Life Technologies). cDNA was synthesized from the extracted mRNA using the Verso cDNA Kit (Thermo Scientific). RT-PCR analysis was then performed with Brilliant III SYBR GREEN Q-PCR (Agilent), with primers (S1 Table) for NESTIN neurofilaments NEFM and NEFL, NURR1, S100B, SAP90 and NT3. The housekeeping gene used was GAPDH. The following amplification parameters were utilized for the q RT-PCR: 10 minutes at 95˚C, 40 cycles of 30 s at 95˚C, 30 s at 55˚C and 30 s at 72˚C, followed by one cycle of 10 s at 95˚C, 5 s at 25˚C, 1 s at 70˚C and 1 s at 95˚C. Results were analyzed using the $2^{-\Delta Ct}$ method relative gene expression to GAPDH.

8. Immunocytochemistry

MSCs were assayed as described preciously [41]. Nuclei were counterstained with DAPI. The primary and secondary antibodies used are shown in S2 Table. The conditions were maintained in negative controls. The dishes were examined under a fluorescence microscope (NIKON ECLIPSE Ti-s). ImageJ software (National Institute of Health) was used to pseudo-color images, adjust contrast, and add scale bars.

9. Synaptic vesicle accumulations

To visualize synaptic vesicle accumulations, after 18 days of neural induction, MSCs from all sources were loaded with 4 μM styryl dye SynaptoRed C2 {4-[6-[4-(Diethylamino) phenyl]-1,3,5-hexatrien-1-yl]-1-[3-(triethylammonio) propyl] pyridinium dibromide, FM 4–64 molecular probes, Tocris Bioscience} in depolarizing extracellular solution (80 μM) during 120 seconds. After loading, cells were washed witch HBSS 1X during 5 min. Cells were imaged immediately under fluorescence microscope NIKON ECLIPSE Ti-s.

10. Statistical analysis

All results are based on at least three independent experiments and are expressed as mean ± SEM or SD for three MSCs donors in each group. Statistical significance for PDT and CTCF analysis was determined using t-student test. In RT-PCR analysis of relative expression and area and neurite outgrowth measurements one-way ANOVA was used, followed by a Bonferroni multiple comparison test using Prism5 software (GraphPad, La Jolla, CA).

Results

1. Characterization of MSCs Isolated from different tissues

a) Morphology. Once in culture, MSCs from all sources were relatively homogeneous in morphology with a characteristic fibroblastic-like morphology when attached to culture plastic dishes (S1 Fig)

b) Immunophenotype. MSCs were tested for analysis of expression of different markers by flow cytometry. Results (S2 Fig) indicate that cells from all tissue sources were negative for hematopoietic marker (CD34), leucocitic markers (CD11b, CD19 and CD45), and HLA-DR (Human Leukocyte Antigen). In turn, were positive for specific MSCs markers (CD73, CD105) and cell adhesion markers (CD29, CD90). In all different donors and tissue origin, the above phenotype was consistent, thus confirming the MSCs phenotype previously described [42–45].

c) Adipogenic, chondrogenic and osteogenic differentiation. MSCs isolated from AT, BM, Skin and UC were capable to differentiate into adipogenic (S3 Fig), chondrogenic (S4 Fig)

and osteogenic (S5 Fig) lineages which is consistent with the minimal characterization criteria of MSCs [42].

d) Proliferation. As shown in the growth curve in Fig 1A, MSCs from all sources, after undergoing an adaptation period to culture conditions during the first 4 days, enter to a logarithmic phase, and later on day 8, exhibit contact inhibition and reach the plateau phase. The Population Doubling Time (PDT) calculated at day 6 (Fig 1B) showed that UC-MSC had reaches a larger population in less time (±72.77 hours), which means that has greater proliferation capacity, followed by AT-MSC (±78.37 hours) and SD-MSC (±82.11 hours) which did not show significant differences. Moreover, BM-MSC show the lowest PDT (±150.52 hours) which was statistically significant.

2. Assessment of mesenchymal stem cell neurogenic potential

Human MSCs previously characterized from the all sources were subjected to neuronal induction medium during 18 days. To investigate whether AT-MSC, BM-MSC, SD-MSC and UC-MSC exhibited neurogenic differentiation capabilities, we compared morphology changes and expression of neural markers at mRNA and protein levels during neural induction.

2a) Morphologic change after neural induction. To assess MSCs neurogenical potential, we analyzed the morphological change in neural induced MSCs. Following 18 days in neural induction medium [39], MSCs from different sources (AT, BM, Skin, UC) changed their morphology from flat, spindle-shaped cells to neural-like cells which included retraction of the cytoplasm towards the nucleus and several cytoplasmic extentions, similar to those exhibited by positive control neural stem cells (Fig 2A–2E). This change is more distinguishable in SD-MSC and AT-MSC (Fig 2A and 2E), which also reduced their sized, confirmed by the quantification of area (Fig 2K) and number of visible neurite outgrowth (Fig 2L).

2b) Expression of neural markers. Our results of immunofluoresence analysis confirmed the expression of nestin at day 5 of neural induction (Fig 3A–3D). Since nestin is a progenitor marker, its expression was analyzed at early stages of neural induction (day 5) and compared with at a later stage (day 18, Fig 3E) by RT-PCR. Our results showed that nestin expression at day 5 was higher as compared with expression at day 18 (Fig 3E). Further, nestin expression was considerably higher in AT-MSC as compared to BM-MSC, SD-MSC and UC-MSC.

MSC from all sources express βIII tubulin, as measured by immunocytochemistry (Fig 4A–4L), as well as the dopaminergic marker tyrosine hidroxilase, estructural marker βIII tubulin and synaptic marker synapthophysin, at 18 day of neural induction. At day 18 of neural induction the expression of neurofilament genes *NEFL* and *NEFM* was higher in AT-MSC and SD-MSC, respectively, compared to that of BM-MSC and UC-MSC. Moreover, AT-MSC showed highly superior gene expression of the dopaminergic neuron marker NURR1, astrocyte marker S100B and neurotrophic factor NT-3, which was statistically significant (Fig 4M).

We additionally detected the labeling of FM 4–64 (synaptored C2) that becomes fluorescent when incorporated into plasma membrane, used to follow up synaptic activity in induced MSC from all studied sources (Fig 5) and quantified, showing that AT-MSC has higher fluorescence intensity against BM, Skin and UC (Fig 5E).

Discussion

The objective of this study was to provide a comparison of the capability of MSCs isolated from several human tissues, to differentiate under *in vitro* conditions to neuron-like cells, evidenced by morphological changes and by the expression of neural markers.

It is well known that several donor characteristics such as age, gender, underlying medical conditions and/or use of concomintant drugs, affect the functional properties of MSCs [46–

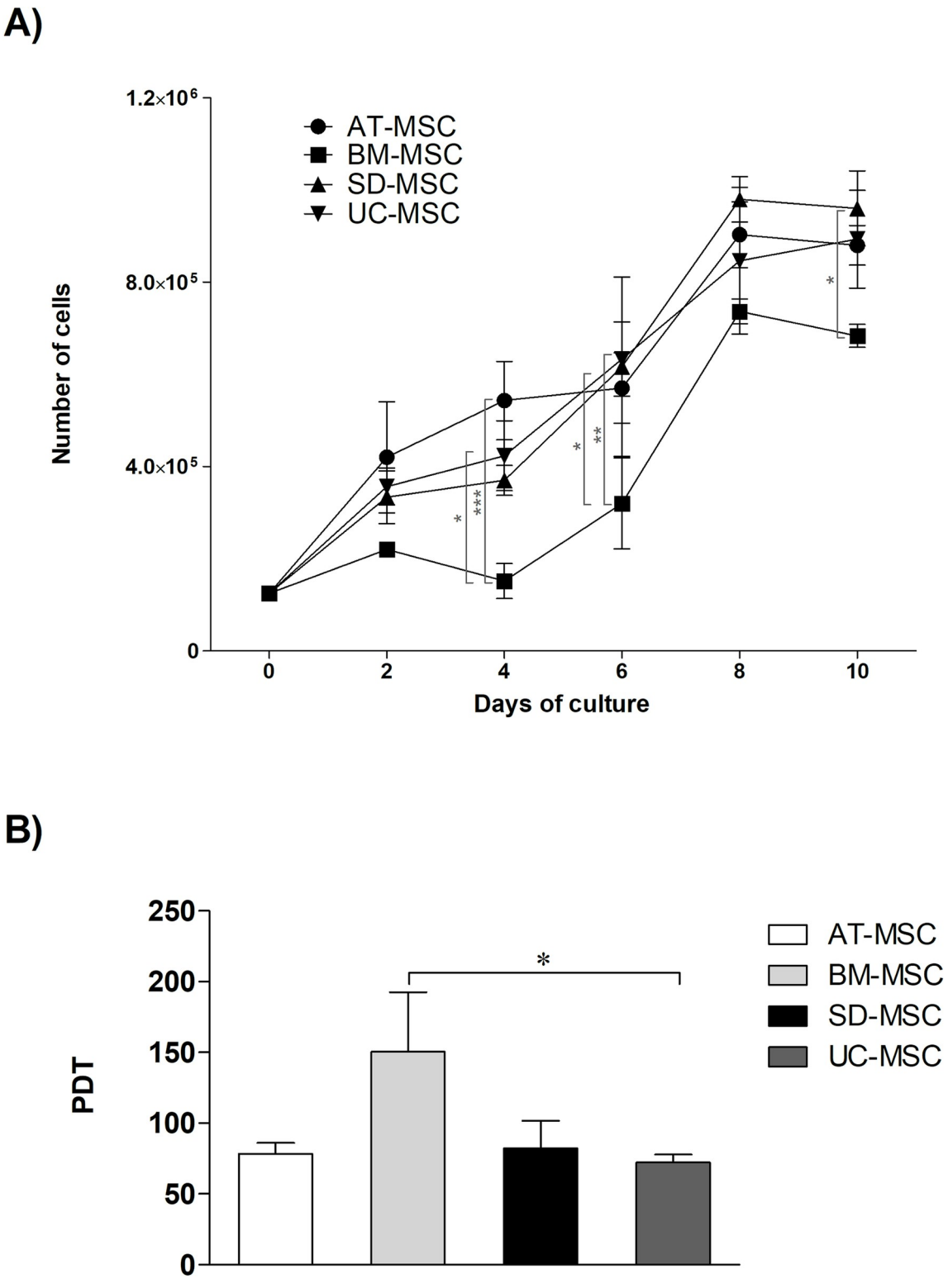

Fig 1. Proliferation of MSCs from different sources. Proliferation capability of AT-MSC, BM-MSC, SD-MSC and UC-MSC. **A)** Growth curve and **B)** Population doubling time. ns P> 0.05, * P <0.05, ** P <0.01, *** P <0.001.

https://doi.org/10.1371/journal.pone.0213032.g001

49]. Therefore, to minimize the putative effect of the above mentioned factors on MSCs neural differentiation capacity, in this study, human tissues were solely obtained from healthy donors of similar age (Table 1).

The results reported here suggest that MSCs isolated from adipose tissue, bone marrow, skin and umbilical cord tissue share common cell surface epitopes as well as an ability to undergo multilineage mesenchymal differentiation (S2–S5 Figs).

Proliferative of MSCS isolated and *ex vivo* expanded from different tissue sources

The proliferative capacity of AT-MSC and UC-MSC (Fig 1), was higher than SD-MSC and BM-MSC. These differences suggest a cell culture heterogenicity, including a variable proportion of self renewing cells, versus lineage-commited cells in different tissue source stromal cell compartment [50,51]. These results could be significant in the election of a tissue source of MSCs, intended to be used in cell-based therapies, which need a viable and ample number of cells to be procured in less time in order to achieve a successful clinical outcome [52].

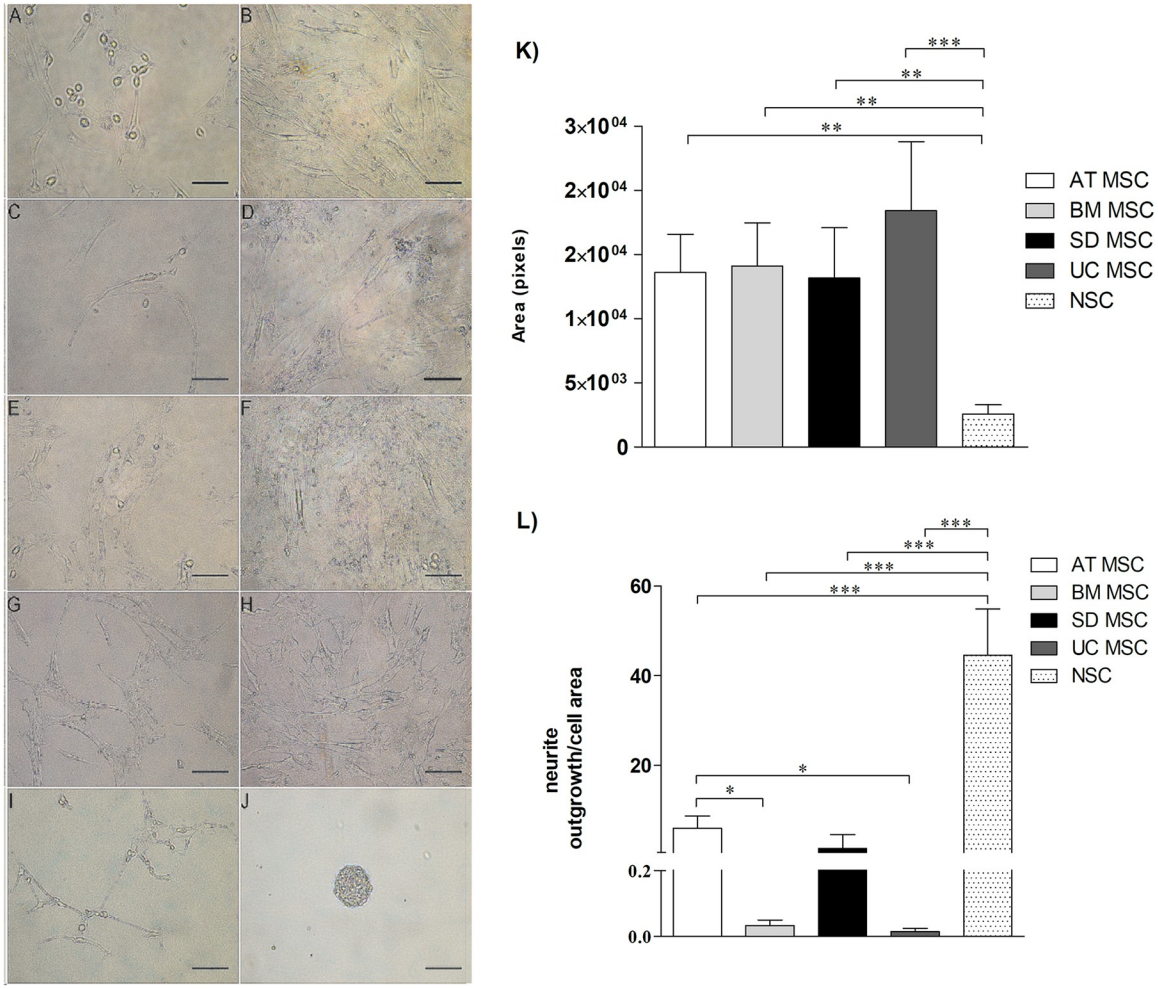

Fig 2. Changes in MSC morphology. Changes in MSC morphology after 18 days of neural differentiation: **(A, B)** AT-MSC, **(C, D)** BM-MSC, **(E, F)** SD-MSC, **(G, H)** UC-MSC, **(I, J)** Positive control (NSC). Scale bar 100 μm. Induced MSC (**A, C, E, G**) adopt neural-like morphology as well as cytoplasm retraction towards the nucleus, which is more notorious in A y E compared to negative controls (**B, D, F, H**) and were quantified in **(K)** Area (pixels) and **(L)** Number of cells with visible neurite outgrowth. Data represents means ± SEM of 3 separate experiments. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

https://doi.org/10.1371/journal.pone.0213032.g002

Assessment of the capacity of MSCs from diverse sources to differentiato into neural-like cells

Results of neural induction of MSCs obtained from different human tissue-sources, indicated that after 18 days of exposure to a neural induction medium, MSCs morphology changed from

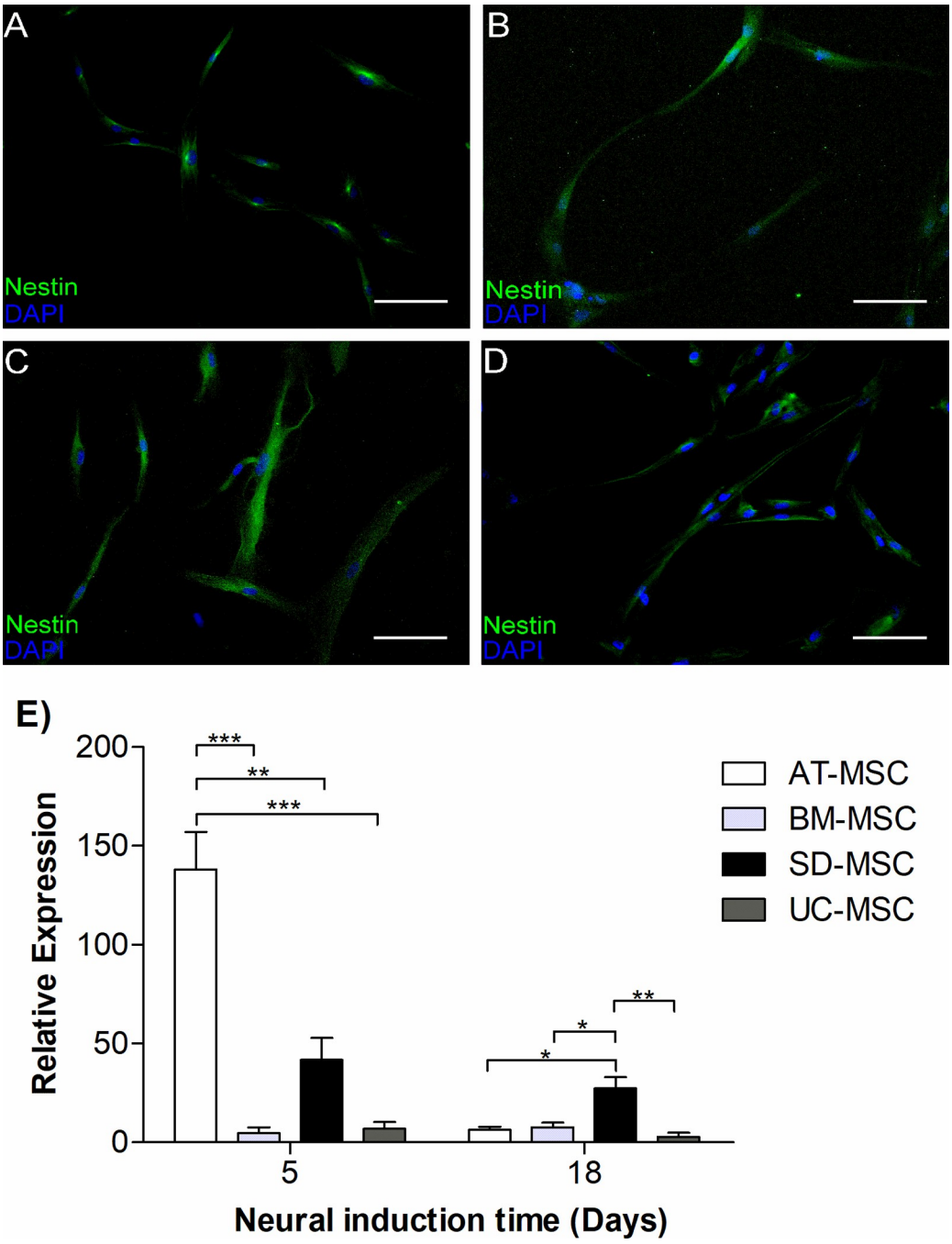

Fig 3. Nestin expression in induced MSC. Nestin relative expression in AT-MSC, BM-MSC, SD-MSC and UC-MSC after neural induction. **(A, B, C, D)** Immunocytochemistry analysis of nestin at day 5 of neural induction; **(A)** AT-MSC, **(B)** BM-MSC, **(C)** SD-MSC, **(D)** UC-MSC. Nestin marker expression (green) and nuclei (Blue), scale bar 100 μm. **E)** Time dependent expression of *nestin* assessed by RT-PCR. Data is presented as an average of three independent patient samples and error bars represent mean ± SEM, ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

https://doi.org/10.1371/journal.pone.0213032.g003

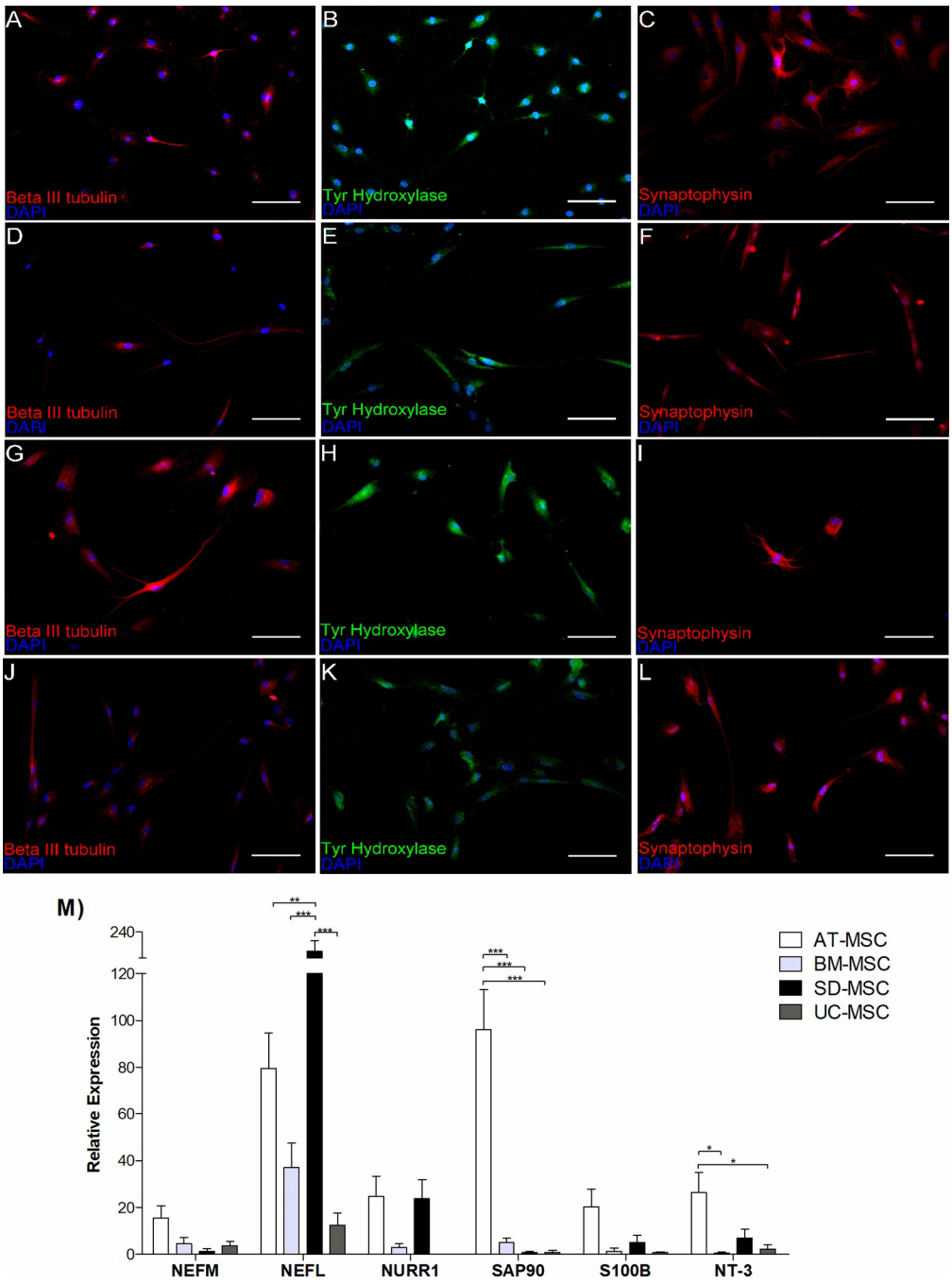

Fig 4. Expression of neural lineage related markers after neural induction. Relative expression of related markers after neural induction in MSC from different sources. **(A-L)** Immunocytochemistry analysis of induced MSC: **(A-C)** AT-MSC, **(D-F)** BM-MSC, **(G-I)** S-MSC, **(J-L)** UC-MSC, showing protein expression of neural specific markers: βIII tubulin (red), tyrosine hydroxylase (green) and synaptophysin (red). Scale bar 100 μm. **M)** Comparative analysis of mRNA expression levels of neural markers, NEFM, NEFL, Nurr1, Sap90, S100b and NT-3. Relative gene expression of each gene were normalized to the expression of the housekeeping gene GAPDH. Data represents means ± SEM of 3 separate experiments. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

https://doi.org/10.1371/journal.pone.0213032.g004

a spindled to a neuron-like shape (Fig 2). Beyond these morphologycal changes, indicative of MSCs neural differentiation, we also studied the onset of a meaningful group of neural

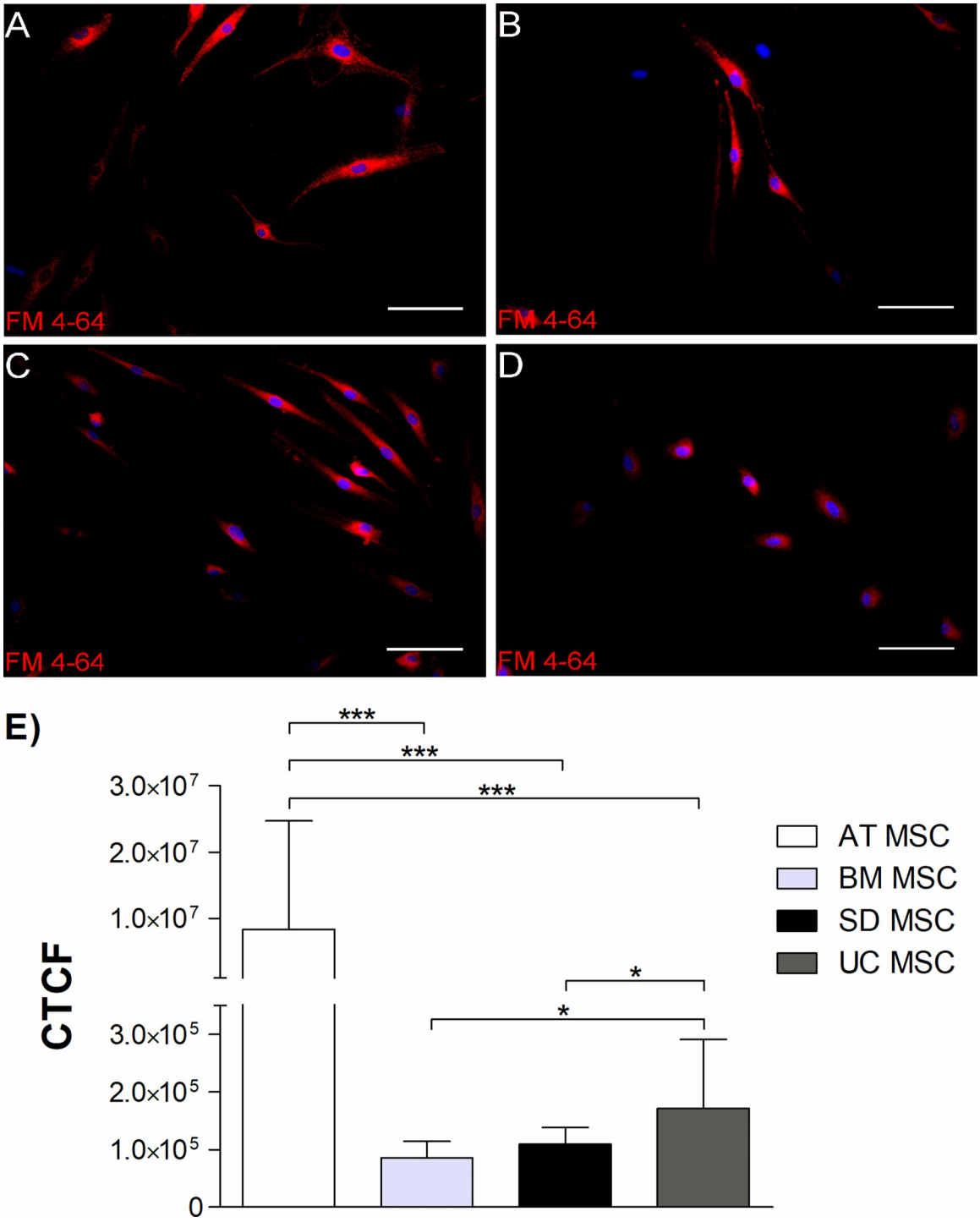

Fig 5. Synaptic vesicle staining after 18 days of neural differentiation. FM 4–64 dye was positive in neural-induced MSC. (A) AT-MSC, (B) BM-MSC, (C) SD-MSC, (D) UC-MSC. Nestin marker expression (green) and nuclei (Blue), Scale bar 100 μm. (E) Corrected Total Cell Fluorescence (CTCF), Data represents means ± SEM of 3 separate experiments. ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

https://doi.org/10.1371/journal.pone.0213032.g005

markers, including *nestin*, *nefm*, *nefl*, *sap90*, *nurr1*, *s100b*, *nt3*, βIII tubulin, tyrosine hydroxylase and synaptophisin (Figs 3 and 4).

The neural induction medium applied during differentiation comprises the use of substances that induce the molecular expression pattern that occurs during adult neurogenesis of neur al stem cells, where neuronal differentiation and maturation occur in three steps: -neural undifferentiated progenitors, -immature neurons expressing early neuronal genes and finally, mature neurons expressing late neuronal genes [53, 54].

To asses this process, we first analyzed nestin expression by MSC, neural stem cell marker that can be considered as a primary evidence of the capability of these cells to generate neural progenitors [55]. The above in turn is sustained by the observed time-dependent expression of *nestin* at day 5, which descreased after 18 days of induction (Fig 3A and 3B).

To evaluate whether MSCs also express a potential to generate immature neurons, we confirmed the expression of βIII tubulin (Fig 4A, 4D, 4G and 4J), an indicator of neural commitmnent [56]. Finally, when cells receive BDNF neurogenic stimulation, they differentiated and acquired the mature phenotype expressing NEFL and NEFM (Fig 4M) which are asossiated with neural maturation [57, 58].

Assesstment of synapse, astrocytes, dopaminergic and neurotrophic markers

In order to demonstrate the protein expression related to synapse formation, we investigated whether AT-MSC, BM-MSC, SD-MSC and UC-MSC were capable of expressing the pre-synaptic protein synaptophysin (Fig 4C, 4F, 4I and 4L), and the post-synaptic protein SAP 90 (PSD95) (Fig 4M). We demostrated that MSCs from all different sources express synaptophysin as evidence of synapsis [59, 60], however AT-MSC showed the highest expression of *sap90*, suggesting that the latter cell type would be the most likely to form a synaptic structure. This results are confirmed in Fig 5A–5E, where AT-MSC show the highest CTCF (Corrected total cell fluorescence) which could probably indicate the presence of functional presynaptic terminals [61].

Results related to the expression of *s100b*, an astrocyte marker, showed that MSCs are capable of differentiating not only into neurons but also into astrocytes. As previously described, adult NSC are specialized astrocytes in others parts of the brain [62]. Astrocytes has the potential to promote neurogenesis in the adult hippocampus [63]. All this considered, and in agreement with our results, we suggest that AT-MSC has higher potential to form astrocytes (Fig 4M).

Moreover, we explored if MSCs were capable to express dopaminergic neuron markers *in vitro*, to considere it as a supplying cell source for the treatment of neurodegenerative conditions like Parkinson disease, in that regard, expression of *nurr1* and tyrosine hydroxylase were analyzed. Accordingly, MSCs from all different sources were capable of expressing both markers, suggesting a committed neuronal phenotype [64].

Additionally, we evaluate neurotrophic potential quantifying the expression of *neurotrophin 3* (*NT-3*) at mRNA level. The above is important since growth-factors-mediate the activation and/or mobilization of endogenous stem cells as well as the reparative action of MSCs [24, 65]. Results show that AT-MSC had the highest expression of NT-3, suggesting that this cell type could exert a better neurotrophic effect *in vitro* (Fig 4M), however, measurement of neurotrophic secretion is still need it to define the role of NT3 in differentiation and trophic effects of neural-induced MSCs.

The above differences in neural marker expression between induced-MSCs could be explained by observing aspects that might affect the neural differentiation potential; these include signalling pathways and transcription factors involved in neuronal fate: the same pathways involved in NSC-neurogenesis are involved in the regulation of MSC chondrogenesis or

osteoblastic differentiation [66–68] which could generate different population of differentiated cells. Besides, it has been demostrated that naïve MSCs already express neural-linked markers [69–70], which could indicate the presence of an heterogeneous population of cells and it is possible that the neural differentiation of MSC *in vitro* are due to the contribution of some neural-committed progenitors already present in the culture rather than the neural differentiation of the whole population [71]. Additionally we have to consider the evidence that showing the expression of proteins typical of nervous tissue in stromal cells, such as cathecolamines [72] neurotrophic factor receptors [73] and/or synaptic proteins [74] which could also generate a different neural marker expression pattern.

In summary, the results of this study indicate that after *in vitro* neural induction, MSCs from all analized tissue sources, slightly differ in morphology, phenotipic characteristics and in their potential to differentiate into neuron-like cells. However, AT-MSC proliferate significantly faster, generated neuron-like cells expressing higher levels of neural markers (Figs 1 to 5). Moreover, previous studies [31, 75] have shown that AT-MSC exhibited an eletrophysiologycal response after neural induction, characteristic of mature-functional neurons and fundamental for signal transmission in the nervous system. Additionaly, adipose tissue is one of the most advantageous sources of MSCs, due to their accessibility and easy of isolation. Finally, AT- MSCs have biological advantages in their proliferative capacity, pattern of secreted proteins (basic fibroblast growth factor, interferon-γ, and insulin-like growth factor-1) and immunomodulatory effects [76], showing as emerging and attractive option for stem cell using therapies.

Conclusion

In the present report, neural regenerative therapy using MSCs obtained from different tisssue sources appears as a feasible and a promissing clinical option for the treatment of neurological affections. From the cell types tested, AT-MSC figure as the most appealing cell source, due to it ease of access and faster proliferation rates. Despite our results represent a novelty comparison between attractive sources of MSCs, there is a need for demostrate a genuine and complete neuronal differentiation, based on those criteria that define a neuronal cell [77] which is only probable by functional assays of synaptic transmission, membrane potential and functional action potential [78]. Also, it is evident that future studies are needed to further optimize and maximize the quality, efficacy and safety clinical use of MSCs [79].

Supporting information

S1 Table. Primers utilized in RT-PCR. Description of primers utilized in the RT PCR analysis, name of the gen, sequence, melting temperature, product size (bp) and database code.
(PDF)

S2 Table. Antibodies utilized in immunocytochemistry. Description of the antibodies utilized in the study. Including name, provider and concentration used in the procedure.
(PDF)

S1 Fig. Fibroblastic-like morphology and adherence to plastic of MSCs from different tissue sources. Fibroblastic-like morphology and adherence to plastic evidence of A) AT-MSC, B) BM–MSC, C) SD-MSC, D) UC-MSC. Scale bar 100 μm.
(PDF)

S2 Fig. Immunophenotype of MSCs from different tissue sources. Histograms showing antigen expression in freshly (%): (A) AT-MSC, (B) BM-MSC, (C) SD-MSC and (D) UC-MSC. From left to right CD19, CD44, CD45, CD90, HLA-DR, CD29, CD73 CD105,

CD73, CD34, CD105, CD11b. Black filled histogram: antigen expression; solid red line: auto-fluorescence control.
(PDF)

S3 Fig. Adipogenic differentiation of MSCs from different tissue sources. Adipogenic differentiation of MSCs from A,B) AT-MSC, B,C) BM–MSC, C,D) SD-MSC, D,E) UC-MSC. Negative controls (B,C,D,E). All were stained with Oil Red O. Scale bar 100 μm.
(PDF)

S4 Fig. Chondrogenic differentiation of MSCs from different tissue sources. Adipogenic differentiation of MSCs from A,B) AT-MSC, B,C) BM–MSC, C,D) SD-MSC, D,E) UC-MSC. Negative controls (B,C,D,E). All were stained with Safranin O. Scale bar 100 μm.
(PDF)

S5 Fig. Osteogenic differentiation of MSCs from different tissue sources. Adipogenic differentiation of MSCs from A,B) AT-MSC, B,C) BM–MSC, C,D) SD-MSC, D,E) UC-MSC. Negative controls (B,C,D,E). All were stained with Alizarin Red. Scale bar 100 μm.
(PDF)

Acknowledgments

We would like to acknowledge the contribution of researchers and professionals of Regenerative Cell Therapy Center in Clinica las Condes. As well we like to thank to Dr. Flavio Carrión, from Universidad Del Desarrollo, for his kind support with the cytometric analysis and facilities.

Author Contributions

Conceptualization: Pablo Caviedes, Ana Maria Vega-Letter, Claudio M. Jofre.

Data curation: Ana Maria Vega-Letter.

Formal analysis: Daniela N. Urrutia, Ana Maria Vega-Letter.

Funding acquisition: Rodrigo Mardones.

Investigation: Daniela N. Urrutia.

Methodology: Daniela N. Urrutia, Pablo Caviedes.

Project administration: Claudio M. Jofre.

Resources: Claudio M. Jofre.

Supervision: José J. Minguell.

Validation: Rodrigo Mardones.

Visualization: Daniela N. Urrutia, José J. Minguell.

Writing – original draft: Daniela N. Urrutia.

Writing – review & editing: José J. Minguell, Claudio M. Jofre.

References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284 (5411):143-7. https://doi.org/10.1126/science.284.5411.143 PMID: 10102814

2. Baraniak PR, McDevitt PC. Stem cell paracrine actions and tissue regeneration. Regen Med 2010; 25 (1):121–43. https://doi.org/10.2217/rme.09.74
3. Gao F, Chiu S, Motan D, Zang Z, Chen L, Ji HL, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 2016; 7: e2062. https://doi.org/10.1038/cddis.2015.327 PMID: 26794657
4. Keilhoff G, Goihl A, Langnase K, Fansa H, Wolf G. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. Eur. J. Cell Biol 2006; 85(1):11–24. https://doi.org/10.1016/j.ejcb.2005.09.021. PMID: 16373171
5. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immun 2008; 8 (9):726–36. https://doi.org/10.1038/nri2395 PMID: 19172693
6. Friedenstein A, Piatetzky-Shapiro I, Petrakova K. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 1966; 16(3):381–90. PMID: 5336210
7. McElreavey K, Irvine A, Ennis K, McLean W. Isolation, culture and characterization of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. Biochem Soc Trans 1991; 19 (1):29S. https://doi.org/10.1042/bst019029s PMID: 1709890
8. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000; 109(1):235–42. https://doi.org/10.1046/j.1365-2141.2000.01986.x PMID: 10848804
9. Zuk P, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13(12):4279–95. https://doi.org/10.1091/mbc.E02-02-0105 PMID: 12475952
10. Toma J, McKenzie I, Bagli D, Miller F. Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells 2005; 23(6):727–37. https://doi.org/10.1634/stemcells.2004-0134 PMID: 15917469
11. Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Eng Part C, Methods 2008; 14(2):149–56. https://doi.org/10.1089/ten.tec.2008.0031 PMID: 18489245
12. La Noce M, Paino F., Spina A, Naddeo P, Montella R, Desiderio V, et al. Dental Pulp Stem Cells: State of the art and suggestions for a true translation of research into therapy. Journal of Dentistry 2014; 42 (7): 761–768. https://doi.org/10.1016/j.jdent.2014.02.018 PMID: 24589847
13. Seeberger K, Eshpeter A, Korbutt G. Isolation and culture of human multipotent stromal cells from the pancreas. Methods Mol Biol 2011; 698:123–40. https://doi.org/10.1007/978-1-60761-999-4_10 PMID: 21431515
14. Mizuno H, Tobita M, Uysal C. Concise Review: Adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells 2012; 30(5):804–10. https://doi.org/10.1002/stem.1076 PMID: 22415904
15. Kim DW, Staples M, Shinozuka K, Pantcheva P, Kang SD, Borlongan CV. Wharton's Jelly-derived mesenchymal stem cells: phenotypic characterization and optimizing their therapeutic potential for clinical applications. Int J Mol Sci 2013; 14(6):11692–712. https://doi.org/10.3390/ijms140611692 PMID: 23727936
16. Brockmann I, Ehrenpfordt J, Sturmheit T, Brandenburger M, Kruse C, Zille M, et al. Skin-derived stem cells for wound treatment using cultured epidermal autografts: clinical applications and challenges. Stem Cells Int 2018; 2018, ID: 4623615. https://doi.org/10.1155/2018/4623615 PMID: 29765411
17. Trohatou O, Roubelakis M. Mesenchymal stem/stromal cells in regenerative medicine: past, present, and future. Cell reprogram 2017; 19(4):217–24. https://doi.org/10.1089/cell.2016.0062 PMID: 28520465
18. Stellavato A, La Noce M, Corsuto L, Pirozzi A, De Rosa M, Papaccio G. Hybrid Complexes of High and Low Molecular Weight Hyaluronans Highly Enhance HASCs Differentiation: Implication for Facial Bioremodelling. Cell Physiol Biochem 2017; 44:1078–1092. https://doi.org/10.1159/000485414 PMID: 29179206
19. Hess D, Borlongan C. Stem cells and neurological diseases. Cell prolif 2007; 41(1):94–114. https://doi.org//10.1111/j.1365-2184.2008.00486.x PMID: 18181951
20. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 2010; 67(10):1187-94. https://doi.org/10.1001/archneurol.2010.248 PMID: 20937945
21. Borlongan C, Glover L, Tajiri N, Kaneko Y, Freeman TB. The great migration of bone marrow-derived stem cells toward the ischemic brain: therapeutic implications for stroke and other neurological disorders. Prog Neurobiol 2011; 95(2):213–28. https://doi.org/10.1016/j.pneurobio.2011.08.005 PMID: 21903148

22. Harach T, Jammes F, Muller C, Duthilleul N, Cheatham V, Zufferey V, et al. Administrations of human adult ischemia-tolerant mesenchymal stem cells and factors reduce amyloid beta pathology in a mouse model of Alzheimer's disease. Neurobiol Aging 2017; 51:83–96. https://doi.org/10.1016/j.neurobiolaging.2016.11.009 PMID: 28056358

23. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. Cell Transplant 2016; 25(5):829–48. https://doi.org/10.3727/096368915X689622 PMID: 26423725

24. Teixeira FG, Carvalho MM, Panchalingam KM, Rodrigues AJ, Mendes-Pinheiro B, Anjo S, et al. Impact of the secretome of human mesenchymal stem cells on brain structure and animal behavior in a rat model of Parkinson's Disease. Stem Cells Transl Med 2017; 6(2):634–46. https://doi.org/10.5966/sctm.2016-0071 PMID: 28191785

25. Syková E, Rychmach P, Drahorádová I, Konrádová Š, Růžičková K, Voříšek I, et al. Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: results of phase I/IIa clinical trial. Cell Transplant 2017; 26(4):647–58. https://doi.org/10.3727/096368916X693716 PMID: 27938483

26. Chung TN, Kim JH, Choi BY, Chung SP, Kwon SW, Suh SW. Adipose-derived mesenchymal stem cells reduce neuronal death after transient global cerebral ischemia through prevention of blood-brain barrier disruption and endothelial damage. Stem Cells Transl Med 2015; 4(2):178–85. https://doi.org/10.5966/sctm.2014-0103 PMID: 25548390

27. Shalaby SM, Sabbah NA, Saber T, Abdel Hamid RA. Adipose-derived mesenchymal stem cells modulate the immune response in chronic experimental autoimmune encephalomyelitis model. IUBMB Life 2016; 68(2):106–15. https://doi.org/10.1002/iub.1469 PMID: 26757144

28. Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, et al. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiol Aging 2012; 33(3):588–602. https://doi.org/10.1016/j.neurobiolaging.2010.03.024 PMID: 20471717

29. Yan ZJ, Hu YQ, Zhang HT, Zhang P, Xiao ZY, Sun XL, et al. Comparison of the neural differentiation potential of human mesenchymal stem cells from amniotic fluid and adult bone marrow. Cell Mol Neurobiol 2013; 33(4):465–75. https://doi.org/10.1007/s10571-013-9922-y PMID: 23478940

30. Zemel'ko VI, Kozhukharova IB, Alekseenko LL, Domnina AP, Reshetnikova GF, Puzanov MV, et al. Neurogenic potential of human mesenchymal stem cells isolated from bone marrow, adipose tissue and endometrium: a comparative study. Tsitologiia 2013: 55(2)101–10. https://doi.org/10.1134/S1990519X13030140 PMID: 23718072

31. Jang S, Cho HH, Cho YB, Park JS, Jeong HS. Functional neural differentiation of human adipose tissue-derived stem cells using bFGF and forskolin. BMC Cell Biol 2010; 16; 11:25. https://doi.org/10.1186/1471-2121-11-25 PMID: 20398362

32. di Summa PG, Kingham PJ, Raffoul W, Wiberg M, Terenghi G, Kalbermatten DF. Adipose-derived stem cells enhance peripheral nerve regeneration. J Plast Reconstr Aesthet Surg 2010; 63(9): 1544–52. https://doi.org/10.1016/j.bjps.2009.09.012 PMID: 19828391

33. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005; 33(11):1402–16. https://doi.org/10.1016/j.exphem.2005.07.003 PMID: 16263424

34. Leite C, Silva NT, Mendes S, Ribeiro A, de Faria JP, Lourenço T, et al. Differentiation of human umbilical cord matrix mesenchymal stem cells into neural-like progenitor cells and maturation into an oligodendroglial-like lineage. PLoS One 2014; 9(10):e111059. https://doi.org/10.1371/journal.pone.0111059 PMID: 25357129

35. Al-Nbaheen M, Vishnubalaji R, Ali D, Bouslimi A, Al-Jassir F, Megges M, et al. Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Stem Cell Rev 2013; 9(1): 32–43. https://doi.org/10.1007/s12015-012-9365-8 PMID: 22529014

36. Roth V. 2006, Doubling Time Computing, http://www.doubling-time.com/compute.php, Oct. 9, 2018.

37. Rebelatto CK, Aguiar AM, Moretão MP, Senegaglia AC, Hansen P, Barchiki F et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med (Maywood) 2008; 233(7):901–13. https://doi.org/10.3181/0712-RM-356 PMID: 18445775

38. Long X, Olszewski M, Huang W, Kletzel M. Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells. Stem Cell Dev 2005; 14(1):65–9. https://doi.org/10.1089/scd.2005.14.65 PMID: 15725745

39. Hermann A, Liebau S, Gastl R, Fickert S, Habisch HJ, Fiedler J, et al. Comparative analysis of neuroectodermal differentiation capacity of human bone marrow stromal cells using various conversion protocols. J of Neurosci Res, 2006; 83(8):1502–14. https://doi.org/10.1002/jnr.20840 PMID: 16612831

40. Delcroix GJ, Curtis KM, Schiller PC, Montero-Menei CN. EGF and bFGF pre-treatment enhances neural specification and the response to neuronal commitment of MIAMI cells. Differentiation 2010; 80(4–5): 213–27. https://doi.org/10.1016/j.diff.2010.07.001 PMID: 20813449

41. Han C, Zhang L, Song L, Liu Y, Zou W, Piao H et al. Human adipose-derived mesenchymal stem cells: a better cell source for nervous system regeneration. Chin Med J 2014; 127(2):329–37. https://doi.org/10.3760/cma.j.issn.0366-6999.20120064 PMID: 24438624

42. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. Cytotherapy 2006; 8(4): 315–17. https://doi.org/10.1080/14653240600855905 PMID: 16923606

43. Ciavarella S, Dammacco F, De Matteo M, Loverro G, Silvestris F. Umbilical cord mesenchymal stem cells: role of regulatory genes in their differentiation to osteoblasts. Stem Cells Dev 2009; 18(8):1211–20. https://doi.org/10.1089/scd.2008.0340 PMID: 19125623

44. Ribeiro A, Laranjeira P, Mendes S, Velada I, Leite C, Andrade P et al. Mesenchymal stem cells from umbilical cord matrix, adipose tissue and bone marrow exhibit different capability to suppress peripheral blood B, natural killer and T cells. Stem Cell Res & Ther 2013; 4(5):125. https://doi.org/10.1186/scrt336 PMID: 24406104

45. Orciani M, Di Primio R. Skin-derived mesenchymal stem cells: isolation, culture, and characterization. Methods Mol Biol 2013; 989:275–83. https://doi.org/10.1007/978-1-62703-330-5_21 PMID: 23483402

46. Katsara O, Mahaira LG, Iliopoulou EG, Moustaki A, Antsaklis A, Loutradis D, et al. Effects of donor age, gender, and in vitro cellular aging on the phenotypic, functional, and molecular characteristics of mouse bone marrow-derived mesenchymal stem cells. Stem Cells and Dev 2011; 20(9):1549–61. https://doi.org/10.1089/scd.2010.0280 PMID: 21204633

47. Nuzzi R, Gunetti M, Rustichelli D, Roagna B, Fronticelli F, Fagioli F, et al. Effect of in vitro exposure of corticosteroid drugs, conventionally used in AMD treatment, on mesenchymal stem cells. Stem Cells Int 2012; 2012:946090. https://doi.org/10.1155/2012/946090 PMID: 22693520

48. Siegel G, Kluba T, Hermanutz-Klein U, Bieback, Northoff H, Schäfer R. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. BMC Med 2013; 11:146. https://doi.org/10.1186/1741-7015-11-146 PMID: 23758701

49. Wyles CC, Houdek MT, Wyles SP, Wagner ER, Behfar A, Sierra RJ. Differential cytotoxicity of corticosteroids on human mesenchymal stem cells. Clin Orthop Rel Res 2015; 473(3):1155–64. https://doi.org/10.1007/s11999-014-3925-y PMID: 25187334

50. Post S, Abdallah B, Bentzon J, Kassem M. Demonstration of the presence of independent pre-osteoblastic and pre-adipocytic cell populations in bone marrow-derived mesenchymal stem cells. Bone 2008; 43(1):32–39. https://doi.org/10.1016/j.bone.2008.03.011 PMID: 18456590

51. Larsen K, Frederiksen C, Burns J, Abdallah B, Kassem M. Identifying a molecular phenotype for bone marrow stromal cells with in vivo bone-forming capacity. J Bone Min Res 2010; 25(4):796–808. https://doi.org/10.1359/jbmr.091018 PMID: 19821776

52. Harris DT. Stem cell banking for regenerative and personalized medicine, Biomedicines 2014; 2(1):50–79. https://doi.org/10.3390/biomedicines2010050 PMID: 28548060

53. Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM. Becoming a new neuron in the adult olfactory bulb. Nat Neurosci 2003; 6: 507–18. https://doi.org/10.1038/nn1048 PMID: 12704391

54. Cardozo A, Gómez D, Argibay P. Neurogenic differentiation of human adipose-derived stem cells: relevance of different signaling molecules, transcription factors, and key marker genes. Gene 2012; 511(2):427–36. https://doi.org/10.1016/j.gene.2012.09.038 PMID: 23000064

55. Wislet-Gendebien S, Leprince P, Moonen G, Rogister B. Regulation of neural markers nestin and GFAP expression by cultivated bone marrow stromal cells. J Cell Sci 2003; 116(Pt 16):3295–302. https://doi.org/10.1242/jcs.00639 PMID: 12840074

56. Abrous D, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. Physiol Rev 2005; 85(2):523–69. https://doi.org/10.1152/physrev.00055.2003 PMID: 15788705

57. Lopez-Picon F, Uusi-Oukari M, Holopainen I. Differential expression and localization of the phosphorylated and non-phosphorylated neurofilaments during the early postnatal development of rat hippocampus. Hippocampus 2003; 13(7):767–79. https://doi.org/10.1002/hipo.10122 PMID: 14620872

58. Lariviere R, Julien J. Functions of intermediate filaments in neuronal development and disease. J of Neurobiol 2004; 58(1):131–48. https://doi.org/10.1002/neu.10270 PMID: 14598376

59. Chindewa R, Lapanantasin S, Sanvarinda Y, Chongthammakun S. Pueraria mirifica, phytoestrogen-induced change in synaptophysin expression via estrogen receptor in rat hippocampal neuron. J Med Assoc Thai 2008; 91(2):208–14. PMID: 18389986

60. Hayase M, Kitada M, Wakao S, Itokazu Y, Nozaki K, Hashimoto N, et al. Committed neural progenitor cells derived from genetically modified bone marrow stromal cells ameliorate deficits in a rat model of

stroke. J Cereb Blood Flow Metab 2009; 29(8):1409–20. https://doi.org/10.1038/jcbfm.2009.62 PMID: 19436312

61. Ryan TA. Presynaptic imaging techniques. Curr Opin Neurobiol 2001; 11(5):544–9. https://doi.org/10.1016/S0959-4388(00)00247-6 PMID: 11595486

62. Götz M, Sirko S, Beckers J, Irmler M. Reactive astrocytes as neural stem or progenitor cells: In vivo lineage, in vitro potential, and Genome-wide expression analysis. Glia 2015; 63(8):1452–68. https://doi.org/10.1002/glia.22850 PMID: 25965557

63. Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. Nat Neurosci 2002; 5(5):438–45. https://doi.org/10.1038/nn844 PMID: 11953752

64. Tatard V, D'Ippolito G, Diabira S, Valeyev A, Hackman J, McCarthy M, et al. Neurotrophin-directed differentiation of human adult marrow stromal cells to dopaminergic-like neurons. Bone 2007; 40(2):360–73. https://doi.org/10.1016/j.bone.2006.09.013 PMID: 17085092

65. Mele L, Vitiello PP, Tirino V, Paino F, De Rosa A, Liccardo D. et al., Changin paradigms of Cranio Facial Regeneration: Current and New Strategies for the Activation of Endogenous Stem Cells. Front Physiol 2016; 7:62. https://doi.org/10.3389/fphys.2016.00062 PMID: 26941656

66. Etheridge SL, Spencer GJ, Heath DJ, Genever PG. Expression profiling and functional analysis of wnt signaling mechanisms in mesenchymal stem cells. Stem Cells 2004; 22: 849–60. https://doi.org/10.1634/stemcells.22-5-849 PMID: 15342948

67. Ling L, Nurcombe V, Cool SM. Wnt signaling controls the fate of mesenchymal stem cells. Gene 2009; 433: 1–7. https://doi.org/10.1016/j.gene.2008.12.008 PMID: 19135507

68. Yanjie J, Jiping S, Yan Z, Xiaofeng Z, Boai Z, Yajun L. Effects of Notch-1 signalling pathway on differentiation of marrow mesenchymal stem cells into neurons in vitro. Neuroreport 2007; 18: 1443–7. https://doi.org/10.1097/WNR.0b013e3282ef7753 PMID: 17712271

69. Montzka K, Lassonczyk N, Tschöke B, et al. Neural differentiation potential of human bone marrow-derived mesenchymal stromal cells: misleading marker gene expression. BMC Neurosci 2009; 10: 16. https://doi.org/10.1186/1471-2202-10-16 PMID: 19257891

70. Tondreau T, Lagneaux L, Dejeneffe M, et al. Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. Differentiation 2004; 72: 319–26. https://doi.org/10.1111/j.1432-0436.2004.07207003.x PMID: 15554943

71. Phinney DG. Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy. Cell Cycle 2007; 6: 2884–9. https://doi.org/10.4161/cc.6.23.5095 PMID: 18000405

72. Maestroni GJ. Neurohormones and catecholamines as functional components of the bone marrow microenvironment. Ann N Y Acad Sci 2000; 917: 29–37. https://doi.org/10.1111/j.1749-6632.2000.tb05370.x PMID: 11268355

73. Labouyrie E, Dubus P, Groppi A, et al. Expression of neurotrophins and their receptors in human bone marrow. Am J Pathol 1999; 154: 405–15. https://doi.org/10.1016/S0002-9440(10)65287-X PMID: 10027399

74. Yamazaki K, Allen TD. Ultrastructural morphometric study of efferent nerve terminals on murine bone marrow stromal cells, and the recognition of a novel anatomical unit: the "neuro-reticular complex". Am J Anat 1990; 187: 261–76. https://doi.org/10.1002/aja.1001870306 PMID: 2321559

75. Anghileri S. Pignatelli Marconi, A., Cifelli P, Galié M, Sbarbati A, et al. Neuronal differentiation potential of human adipose-derived mesenchymal stem cells. Stem cells Dev 2008; 17(5):909–16. https://doi.org/10.1089/scd.2007.0197 PMID: 18564036

76. Li CY, Wu XY, Tong JB, Yang XX, Zhao JL, Zheng QF, et al. Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. Stem Cell Res Ther 2015; 6:55. https://doi.org/10.1186/s13287-015-0066-5 PMID: 25884704

77. Reh TA. Neural stem cells: form and function. Nat Neurosci 2002; 5: 392–4. https://doi.org/10.1038/nn0502-392 PMID: 11976698

78. Scuteri A, Miloso M, Foudah D, Orciani M, Cavaleti G, Tredici G. Mesenchymal Stem Cells Neuronal Differentiation Ability: A Real Perspective for Nervous System Repair?. Current Stem Cell Research & Therapy, 2011; 6, 82–92. https://doi.org/10.2174/157488811795495486

79. Volkman R, Offen D. Concise review: mesenchymal stem cells in neurodegenerative diseases. Stem cells 2017; 35(8):1867–80. https://doi.org/10.1002/stem.2651 PMID: 28589621