Rehabilitation Science
2021; 6(4): 76-82
http://www.sciencepublishinggroup.com/j/rs
doi: 10.11648/j.rs.20210604.14
ISSN: 2637-5931 (Print); ISSN: 2637-594X (Online)

Report

Transdifferentiation of Human Umbilical Cord Stromal Cells to Neurogenic-Like Cells

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To cite this article:

Pu Jiujun, Wang Zhiming, Ma Xiankun, Zhang Hongdian. Transdifferentiation of Human Umbilical Cord Stromal Cells to Neurogenic-Like Cells. *Rehabilitation Science*. Vol. 6, No. 4, 2021, pp. 76-82. doi: 10.11648/j.rs.20210604.14

Received: December 7, 2021; **Accepted**: December 16, 2021; **Published**: December 20, 2021

Abstract: Objective: To investigate the differentiation of human umbilical cord stromal cells into neurogenic cells. Methods: (1) Human umbilical cord stromal cells (HUMSCs) were pre-induced with recombinant human fibroblast growth factor-2 (hbFGF-2, Peprotech, UK) and N^2 (1:100, Invitrogen, USA), immunocytochemistry and Western blot to determine the expression of nestin and fibronectin before and after pre-induction. Western blot to identify Nestin and fibronectin expression before and after pre-induction. (2) The differentiation of pre-induced and un-induced HUMSCs into neurogenic cells was performed, and the value-added rates of both were measured by MTT at different time points, and the morphological differences between the differentiated cells and the differentiated cells were verified at the gene and protein levels. Results:(1) 94.4 ± 2.3% and 6.5 ± 1.2% HUMSCs expressed ectodermal marker-fibronectin and stem cell marker-nestin, respectively, and 62.3 ± 5.2% piHUMSCs showed nestin immunopositive staining after adding FGF2 and N^2 for pre-induction; 51.3 ± 5.9% of cells showed fibronectin immunopositive staining. piHUMSCs showed significantly higher nestin positivity than HUMSCs ($P<0.05$), whereas HUMSCs expressing fibronectin showed significantly higher positivity than piHUMSCs ($P<0.05$). Western blot results were consistent with cellular immunohistochemistry results. The proliferation rate of HUMSCs and piHUMSCs by MTT assay suggested that after 48 h of culture, both HUMSCs and piHUMSCs entered the logarithmic cell growth phase, and the cell expansion rate of HUMSCs was significantly higher than that of piHUMSCs after 72 h and 96 h of culture.(2) The results of RT-PCR experiments indicated that differentiated human umbilical cord neural stem cells expressed The expression of the corresponding neural stem cell marker genes was statistically different ($p<0.05$) than that of undifferentiated umbilical cord stromal cells and terminally differentiated neural cells after induction of fractionation. Conclusion: Umbilical cord-derived stromal cells pre-induced with recombinant human fibroblast growth factor-2 (hbFGF-2, Peprotech, UK) and N^2 (1:100, Invitrogen, USA) were better able to differentiate into neural stem cells with stable expression of cell morphological changes, proteins and genes.

Keywords: Cord Blood-derived Neural Stem Cells, Stroke, Nerve Damage, Nestin, Fibronectin

1. Introduction

Stroke, traumatic brain injury and other neurological injuries and neurodegenerative diseases can cause human hypofunction, disability and even death. At present, rehabilitation medicine still has not effectively solved the problem of neural repair, and the progress of functional recovery is slow or even difficult, mainly because of the difficulty of repairing neural injury. In recent years, studies have shown that transplantation of neural stem cells can promote the recovery of neurological dysfunction. However, nerve cells are permanent cells and cannot be regenerated

after the death of their own injury. Similarly, some studies have shown that neural stem cells may exist in the hippocampal region within the skull itself, but how to stimulate their activity, and the corresponding mechanism and research are unclear, and more and more studies have also confirmed that nerve repair is the only way to promote functional improvement, so exogenous stem cell implantation can accelerate nerve repair and thus improve patient function; this The present study demonstrated that human umbilical cord stromal cells (HUMSCs) have stable neural stem cell morphological characteristics, protein expression and gene expression after induced differentiation into neurogenic stem cells (HUMSCs-NSC), in order to provide experimental theoretical support for clinical applicati.

2. Materials and Methods

2.1. Experimental Cells and Main Reagents

2.1.1. Experimental Cell Source

All procedures for the adoption of this experiment were approved by the Ethics Committee of the Army General Hospital, and umbilical cord tissues were taken from the umbilical cords of healthy full-term parous fetuses at the Army General Hospital, all with the authorized consent of the parents. Stable primary human umbilical cord stromal cells (Human umbilical cord stromal cells HUMSCs) have been obtained from previous experiments.

2.1.2. Primary Reagents

Rabbit anti-nestin (1:400; Chemicon), mouse anti-beta-tubulin isotype III antibody (1:800; Sigma), mouse anti-Fibronectin (1:600; Sigma), mouse anti-GFAP (1:600; Chemicon), and Rabbit anti-GalC (1:600; Chemicon) polyclonal antibody, Recombinant Human Fibroblast Factor-2 (hbFGF-2, Peprotech, UK) and N^2 (1:100, Invitrogen, USA), BCA Protein Assay kit (HyClone, USA), DMEM- F12 medium, (HyClone, USA), fetal bovine serum (HyClone, USA), 0.25% trypsin (HyClone, USA), Neurobasal medium (NB medium, Invitrogen), 20 ng/ml EGF (Epidermal growth factor, EGF, Peprotech, UK).

2.1.3. Main Instruments

Hitachi electron scanning electron microscope (Japan), Fluoview FV300 laser confocal microscope (Olympus), water-jacketed CO_2 incubator (USA). Biogel imaging system (Genius).

2.2. Isolation and Culture Experiments of HUMSCs

Cord blood was collected under aseptic conditions, and cord tissues were removed and stored in D-Hank's solution at 4°C. The umbilical cord tissues were soaked in 75% alcohol for about 30 seconds (sec) on an ultra-clean bench, cut into 1-mm size pieces, and rinsed three times with D-Hank's solution. The tissue was then digested with 0.075% type II collagenase for 18 hours (h) at 37°C, followed by 0.125% 0.125% trypsin/EDTA for 30 minutes (m) at 37°C. The cells were neutralized with DMEM/F12 medium containing 10% fetal bovine serum (FBS). A drop of cell suspension was taken on a hematocrit plate, and the total number of cells in four large compartments was counted under the microscope to calculate cell density (the total number of cells in the four compartments was divided by 4, and the result was multiplied by 104 to be the cell density per ml), and the density was adjusted to 1×106/mL and inoculated with DMEM/F12 containing 10% FBS, 100 kU/L penicillin, and 100 mg/L streptomycin. The cells were cultured in 75 cm^2 T-shaped plastic culture flasks containing 10% FBS, 100 kU/L penicillin and 100 mg/L streptomycin in a 5% CO_2 incubator at 37°C. After 2-3 days, the cells were changed in full volume, and after discarding the non-adherent cells, the cells were changed in half volume every 3-4 days. When the primary culture cells grew to 80% fusion, the culture medium and suspended cells were removed and rinsed 3 times with 3 mL of .01 mol/L pre-cooled PBS. After discarding the PBS, add 0.25% trypsin solution about 0.5mL and leave it at room temperature for 2~3min, observe the change of cell morphology under an inverted microscope; when the cells shrink into a sphere and some of them start to detach from the wall of the vessel, immediately add 2~3 drops of FBS to terminate the digestion reaction; then blow gently with a pipette repeatedly to make the adherent cells fall off. Transfer the digested cell suspension into a centrifuge tube, centrifuge for 5 min at 1000 rpm at room temperature, remove the supernatant, resuspend the cells with culture medium, gently blow to form a single cell suspension, inoculate in a new culture flask or culture plate at 1:3 passages; incubate at 37°C in a 5% CO_2 saturated humidity incubator.

2.3. Pre-induction and Transdifferentiation of HUMSCs to Neural Stem Cells and Cell Immunochemical Experiments for Identification

2.3.1. Pre-induction Experiments

The cells were digested with 0.125% trypsin/EDTA, collected, and 2×106 HUMSCs isolated by digestion were grown in 75 mm^2 culture flasks with DMEM/F12 medium, 5 ng/ml recombinant human fibroblast growth factor-2 (hbFGF-2, Peprotech, UK) and N^2 (1:100, Invitrogen, USA) were incubated at 37°C for 3 days in an incubator containing 5% CO_2 for pre-induction.

2.3.2. Transdifferentiation Assay

The pre-induced HUMSCs were digested with 0.125% trypsin/EDTA, and then 1×105 cells/ml were grown in 25 cm^2 low-adhesion culture flasks with Neurobasal medium (NB medium, Invitrogen), 20 ng/ml EGF (Epidermal growth factor, EGF, Peprotech, UK) and N^2 (1:100, Invitrogen, USA). bFGF and EGF were added every 3-4 days and the NB medium was changed once a week. after 4-5 days, suspended neurospheres appeared. When the average diameter of neurospheres reached 100 μm, passaging was performed. The neurospheres were continued to be expanded for 4-6 weeks (passaged for 3-4 generations) before proceeding to the next step of induction.

2.4. Growth and Proliferation Rate of HUMSCs and piHUMSCs

MTT colorimetric assay was used to detect the growth viability of HUMSCs and piHUMSCs. Each group of cells was inoculated in 96-well culture plates at 1×104/well, and 20μl of 5mg/ml MTT (Sigma, USA) solution was added to each well, and incubation was continued at 37°C for 4h, washed with PBS solution, then 200μl DMSO was added and shaken for 10min to fully dissolve the crystals. The absorbance was measured with an ELISA (λ=570 nm). The growth curve was plotted using time as the horizontal coordinate and the average absorbance of 6 wells as the vertical coordinate.

2.5. Immunohistochemical Identification of Cells

The culture medium was removed from the culture plate, rinsed 3 times with 0.01 mol/L PBS, fixed with 4% paraformaldehyde for 30 min; primary antibodies diluted with serum diluent were added: rabbit anti-nestin (1:400; Chemicon), mouse anti-Fibronectin (1:600, Sigma) were incubated overnight at 4°C in a wet box, and the primary antibodies were removed. 0.01 mol/L PBS was rinsed 3 times; secondary antibodies Alexa Fluor 594-labeled goat anti-mouse IgG (1:200) and Alexa Fluor 488-labeled goat anti-rabbit IgG (1:200) were added dropwise, incubated at room temperature for 2 h. After removal of secondary antibodies, 0.01 mol/L PBS was rinsed 3 times; Hoechst 33342 (2 μg/ml) The cell nuclei were re-stained; then the slices were sealed with VECTASHIELD® Mounting Medium and observed by Fluoview FV300 (Olympus) laser confocal microscope. A negative control test was also set up: antibody dilution was used instead of primary antibody, and the result was negative if no color was shown under the microscope.

2.6. Western Blot Assay

2.6.1. Sample Protein Determination Experiment and Protein Electrophoresis

The total protein content of the samples was determined using Bio-Rad's BCA Protein Assay kit according to the steps described in the kit. Prepare 10 ml of 12% isolation gel: perfuse the isolation gel with 5% concentrated gel, prepare and sample the sample, take 10 ul of the adjusted concentration, add 10 ul of 2×Loadding Buffer, mix well, boil for 8 min at 95°C, cool rapidly, centrifuge for 1 min (10000 rpm) (the protein marker was prepared in advance with 2×Loadding Buffer). Loadding Buffer and store at -20°C, then remove before use). Add 20ul of sample to each well and sample in equal amounts. 2.5h of electrophoresis at constant pressure of 120v until the bromophenol blue migrates to the bottom of the gel. The gel, filter paper and nitrocellulose membrane were put into the transfer buffer for 15 min. -Nitrocellulose membrane-gel-filter paper-sponge pad sequence, and drain the air bubbles between the gel, filter paper and fiber membrane. Put the transfer clip into the transfer tank according to the correct polarity direction (the side with nitrocellulose membrane to the positive pole), add the transfer buffer, connect the electrode, turn on the power, and turn on the power at 4°C, 28V for 5h. Turn off the power, remove the transfer film, and mark the positive and negative sides. The transfer membrane was stained in Lixin Red S staining solution for 5-10 min at room temperature, and the membrane was rinsed in deionized water to position the protein molecular weight standard reference, and the position of the standard protein was marked with a pencil. Continue to soak in deionized water for about 10 min to completely decolorize the transfer membrane for the following immunoassay method.

2.6.2. Immunostaining

The membrane is rinsed 3 times with TBST for 5 min each time. The membrane is closed with 10% skim milk powder TBST blocking solution for 2 h. The membrane is rinsed 3 times with TBST for 5 min each time. The primary antibody, rabbit polyclonal antibody to BDNF (1:200), is added and incubated overnight at room temperature with antibody dilution buffer. Add HRP-labeled sheep anti-rabbit IgG (1:5000), also with antibody dilution buffer, and incubate at room temperature for 2 h. Aspirate the liquid from the nitrocellulose membrane and place the protein side up. Take 3 ml of double-distilled water, add one drop each of liquid A and liquid B from the ECM kit to each ml, mix well and add to the membrane, shake to distribute it evenly on the membrane surface, and react in a dark room for 5 min. remove the test membrane, aspirate the liquid from the membrane, place the membrane in an X-ray box, cover the nitrocellulose membrane with plastic wrap, carefully squeeze out the air bubbles between the test membrane and the plastic wrap, and finally cut an X film of comparable size and place it on the plastic wrap. Finally, cut an X film of comparable size and place it on the plastic film, close the X-ray box, and expose it for 30-60 seconds. Do not let the film and the film slide after contact. Develop in developer for 1 min, fix in fixer for 3 min, and let dry. Use β-Actin as internal reference control: rinse the nitrocellulose membrane with Stripping Buffer for 30min (50°C), rinse the membrane 3 times with TBST for 10min each, and again as above (primary antibody is β-Actin, 1:400; secondary antibody is HRP-labeled goat anti-mouse IgG (1:5000). The bands of each protein region of the gel after SDS-PAGE and the positive bands after Western blotting were imaged using the Biogel Imaging System.

2.7. Real-time PCR to Detect the Expression of Some Functional Genes in Stem Cells

The sequences of the relevant functional genes tested are as follows.

FN1 (Fibronectin):
5’-GAG ATC AGT GGG ATA AGC AGC A-3’;
5’-CCTCTTCATGACGCTTGTGGA-3’
NES (Nestin):
5’-TGG CTC AGA GGA AGA GTC TGA-3’;
5’-TCCCCCATTTACATGCTGTGA-3’
OCT-4 (Octomer-binding transcription factor 4):
5’-GTATTCAGCCA ACGACCATC-3’;

5’-CTGGTTCGCTTTCTCTTTCG-3’
OTX1 (Orthodenticle homolog 1):
5’-CACTAACTGGCGTGTTTCTGC-3’;
5’-AGGCGTGGAGCAAAA TCG-3’
SOX1 (Sex determining region Y-box 1):
5’-GCCCAGGAGAACCCC AAG-3’;
5’-CGTCTTGGTCTTGCGGC-3’
Neurog2 (Neurogenin 2)
5’-CGCATCAAGAAGACCCGTAG-3’;
5’-GTGAGTGCCCAGATGTAGTTGTG-3’

Total RNA extraction: cells were lysed with Trizon and RNA was extracted according to the procedure provided by the manufacturer (Invitrogen), 1ul of extracted RNA was aspirated and diluted 10 times with DEPC water, and the RNA concentration was measured on a Nanodrop, 3μg of RNA was taken according to the kit First Strand cDNA Synthesis Kit (GeneCopoeia). Synthesis Kit (GeneCopoeia), reverse transcribed into cDNA, and real time-PCR was performed on a 96-well polypropylene plate on an iQ5 Real Time PCR Detection System (Bio-Rad), with primer sequences. The reaction system was 20 μl per well, containing 2 μl of 1:5 sample cDNA, primer final concentration of 0.4 μM, and 2×AllinOneTM Q-PCR Mix (GeneCopoeia), set program: pre-denaturation: 95°C for 10 min, denaturation 95°C for 10 s, annealing 57°C for 20 s, extension 72°C for 15 s, 40 cycles. Curve analysis: 72°C~95°C temperature interval 0.5°C time interval 6s/time, annealing: 30°C 30s. all PCRs were performed with 3 wells and GAPDH was used as internal reference. Data analysis: In any cycle of PCR, samples were considered positive when the fluorescence intensity value was greater than the threshold value calculated by the software that comes with the instrument. mRNA expression difference fold was expressed as 2-ΔΔCT (ΔCT was obtained by subtracting the CT value of GAPDH from the CT value of the tested gene, and ΔΔΔCT was obtained by subtracting the CT value of the negative control BMSCs from ΔCT). HUMSCs, HUMSC-NSCs, and HUMSC-NSCs, and ultimate differentiated HUMSC-NSCs were taken six times, and each specimen was measured in relative replicates three times to take the average as the mRNA expression level.

2.8. Statistical Methods

The statistical treatment was performed using SPSS13.0 software. The mean-land standard deviation (.1$) was used for normally distributed measures.

3. Results

3.1. Induced Differentiation of HUMSCs to Neural Stem Cells

As shown in Figure 1, 3 d after the pre-induction by adding hbFGF-2/N^2, the cell cytosol started to shrink, the morphology became elongated, and the cell poles became elongated and arranged parallel to each other. At this time, the pre-induced HUMSCs (pre-induced, piHUMSCs) were placed into the neural stem cell medium and cultured. 1-2 d later, the piHUMSCs started to aggregate. After 3-4 d of culture, many neurosphere-like cells began to be suspended in the medium in the form of mulberry embryos. After 7-9 d, most of the neurosphere-like cells were more than 100 μm in size and had a round spherical shape with strong cytosolic refractive index. These neurosphere-like cells were able to expand in vitro for at least two months without changing their morphology. The proliferation of neurosphere-like cells was good, with a mean doubling time of 46.5 ± 1.2h.

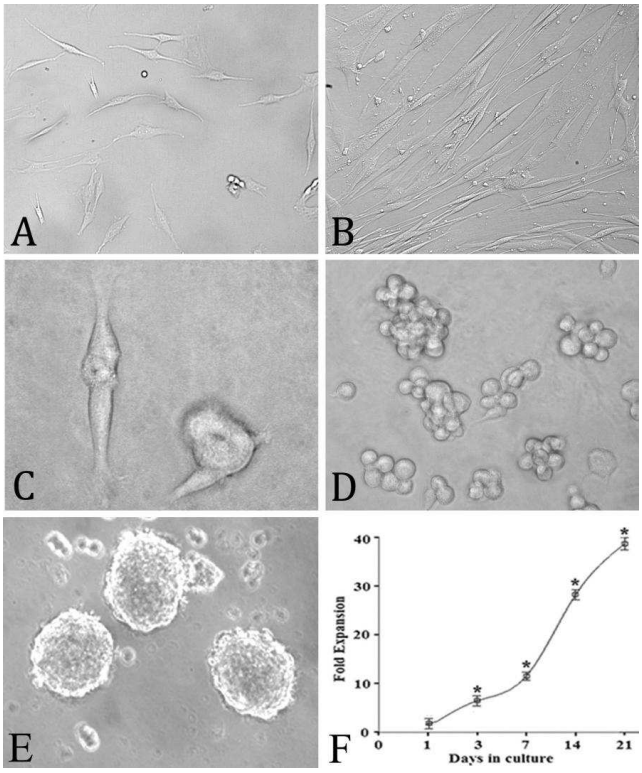

Figure 1. *Transdifferentiation of HUMSCs into NSCs. A: the cells bodies begin to elongate 3 days after plated in the medium supplementing with N^2 and FGF2; B: the induced cells elongate became parallel with each other, and most of the hWJ-MSCs retracted their cytoplasm and developed markedly elongated cell bodies; C: the induced cells began to aggregate after being re-plated in NSC medium; D: Many small spheres of floating cells appeared within 3–4 days after conversion. Fluorescent microcopy, Scale bar shown in A, A, B =50μm; D =25μm; C = 12.5μm.*

3.2. A Comparative Study Between piHUMSCs and HUMSCs

As shown in Figure 2, 94.4 ± 2.3% and 6.5 ± 1.2% of HUMSCs expressed mesodermal marker-fibronectin and stem cell marker-nestin, respectively, while in piHUMSCs, 62.3 ± 5.2% of cells expressed nestin, while only 51.3 ± 5.9% of the cells expressed fibronectin positive staining. In contrast, nestin expression was significantly higher in piHUMSCs than ($P<0.001$) HUMSCs, whereas fibronectin expression was significantly higher in piHUMSCs than HUMSCs ($P<0.001$). Western blot analysis further confirmed the immunohistochemical results.

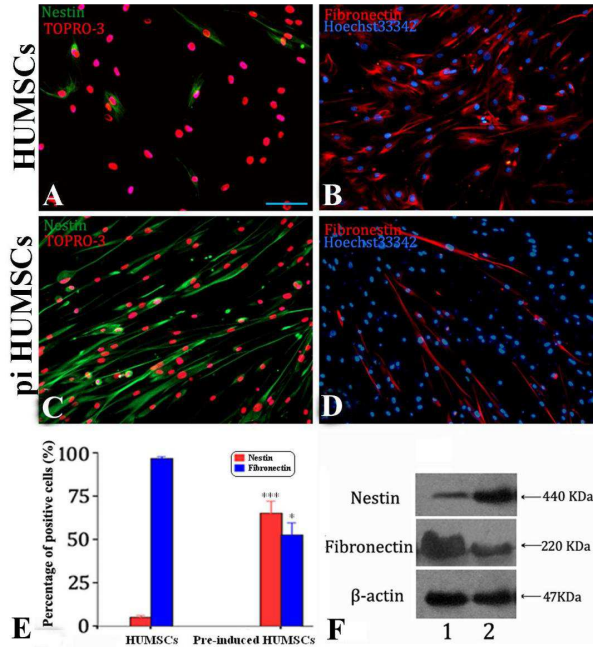

Figure 2. *The expression of fibronectin and nestin in HUMSCs and piHUMSCs. Immuno -reactive staining of HUMSCs. A: Nestin (+) cells in HUMSCs (green); B: Fibronectin (+) cells in HUMSCs (red); C: Nestin (+) cells in piHUMSCs (green); D: Fibronectin (+) cells in piHUMSCs (red) E: Quantification of the percentage of cells immunopositive for nestin and fibronectin in HUMSCs and piHUMSCs. F: Western blot analysis of the protein expressions of nestin and fibronectin in HUMSCs and piHUMSCs. Scale bar shown in A, A-C = 100μm, D = 50μm.*

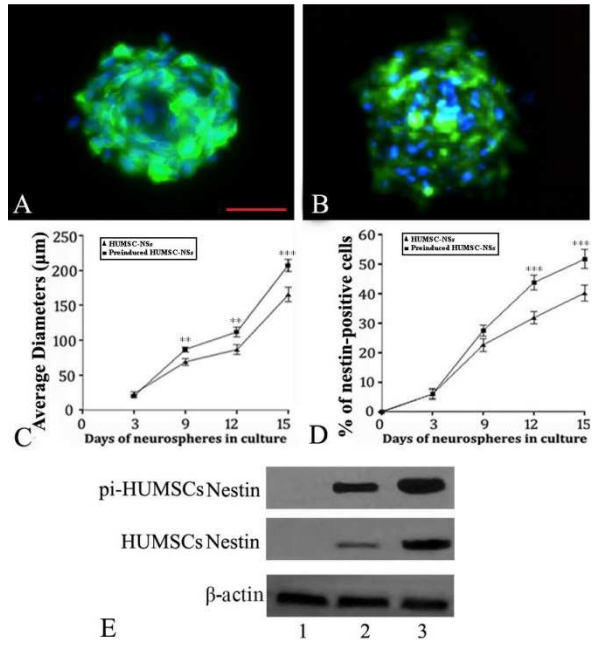

Figure 3. *The comparison of the differentiation efficiencies of HUMSCs and piHUMSCs into neurospheres. A-B: nestin-positive staining (green) of neurospheres differentiated from HUMSCs (A) and piHUMSCs (B). C-D: Quantification of the average diameters (C) and the percentage (D) of cells immunopositive for nestin in neurospheres differentiated from HUMSCs and piHUMSCs. E: Western blot analysis of the protein expressions of nestin in neurospheres differentiated from HUMSCs and piHUMSCs. Scale bar shown in A, A-B = 50μm.*

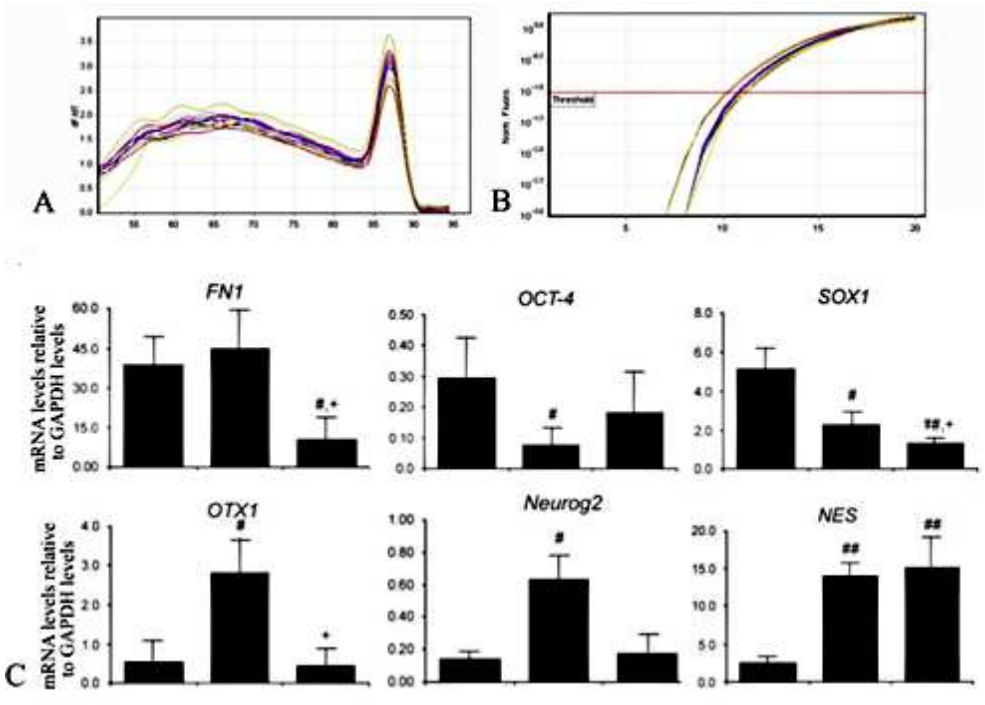

Figure 4. *Quantitative real-time RT-PCR analyses of genen expression in HUMSCs, HUMSC-NSCs and differentiated HUMSC-NSCs. (A) Representative real-time RT-PCR analysis using the LightCycler® technique. Plot of the fluorescence versus the cycle number obtained from SYBR Green detection of serially diluted FN1 mRNA (encoding fibronectin) (B) tomelting curve analysis showing the specificity of the amplified PCR product (right). (C) Quantitative real-time RT-PCR analyses of mesodermal genes (FN1), proneural genes (SOX1, OTX1, Neurog2), NSC marker genes (NES), as well as OCT-4 as a marker for pluripotency in HUMSCs, HUMSC-NSCs and differentiated HUMSC-NSCs, respectively. Expression levels are expressed relative to the housekeeping gene GAPDH. #, P<0.05; ##, P<0.01 when compared to mRNA levels in HUMSCs; +, P < 0.05; ++, P < 0.01 when compared to mRNA levels in HUMSC-NSCs.*

As shown in Figure 2: neurospheres induced by HUMSCs and piHUMSCs both expressed nestin positive staining, and by detecting the change in diameter of neurospheres induced by HUMSCs and piHUMSCs at different time points after induction, it was found that neurospheres induced by piHUMSCs proliferated more rapidly. MTT method was used to detect the proliferation rate of HUMSCs and proliferation rate of piHUMSCs. It was seen that after 48h, both HUMSCs and piHUMSCs entered the logarithmic growth phase of cells. The cell expansion rate of HUMSCs was significantly higher than that of piHUMSCs after 72h and 96h hours of culture (Figure 3). The mean diameter of neurospheres was statistically significantly larger than that of neurospheres induced from iHUMSCs at 9, 12 and 15 days after induction. The nestin positive expression rate was detected on day 7 after induction, and it was found that both neurospheres induced by HUMSCs and piHUMSCs expressed nestin immunopositivity, but the nestin positivity rate of neurospheres induced by piHUMSCs was significantly higher than that of neurospheres induced by HUMSCs. Western blot further validated the immunohistochemical results.

3.3. Expression of Some Functional Genes in Stem Cells

Quantitative analysis of partial functional gene expression revealed that the levels of FN1 were not significantly different between HUMSCs and HUMSC-NSCs, but the expression levels of SOX1, OCT-4 were significantly lower in HUMSC-NSCs. On the other hand, HUMSC-NSCs acquired partial gene expression of stem or precursor cells. the expression levels of Neurog2 and NES in HUMSC-NSCs were 4.5-77.1-fold higher than those in HUMSCs. the expression of OTX-1 was significantly higher in HUMSC-NSCs, indicating the "After differentiation of HUMSC-NSCs to neuronal cells, OTX-1 expression decreased again. The expression level of FN1 was significantly lower in differentiated HUMSC-NSCs compared to HUMSCs and HUMSC-NSCs. Moreover, the levels of SOX1, Neurog2 and NES were also significantly decreased.

4. Discussion

Stroke is currently the most lethal and disabling disease, and survivors place a huge burden on their families and society [1, 2]. The main cause of stroke is brain tissue damage, resulting in neuronal degeneration, necrosis or apoptosis. [3] Various neurological dysfunctions occur as a result of stroke. However, the number of neural stem cells produced is small and insufficient to repair brain injury [4]. In vitro transplanted neural stem cells have the ability of unlimited proliferation and multidirectional differentiation, etc. Chrostek found that they can also be used for neurological recovery after stroke, and many animal experiments and preclinical experiments have also confirmed that neural stem cells can be transplanted to improve neurological function, such as in stroke, spinal cord injury, cancer, etc [5-7]. The main mechanisms may be the secretion of nutritional neurological factors, reduction of neuroinflammatory response, and promotion of neural reconstruction, etc. Currently, transplantation can be done by other means such as injury site transplantation, intracerebroventricular transplantation, or intravascular input, but the specific mechanisms regulating migration repair are still not fully understood [8, 9], and some studies have confirmed that it may be related to the environment in which the neural stem cells are located [10].

Neural stem cell transplantation is considered to be one of the most promising therapeutic modalities [11]; currently, the sources are diverse, mainly induced pluripotent stem cells, such as dental pulp stem cells, and directly isolated and cultured, such as human umbilical cord stromal cells and embryonic neural stem cells [12]. Because of the multiple sources, the heterogeneity after differentiation is also of great concern [10], i.e., whether to differentiate the neural cells we need to reconstruct the injured area; we used human umbilical cord stromal cells for our study. The human umbilical cord stromal cells we used for the study are easy to obtain because of their wide source and also have good consistency; pre-current research on the treatment of neural stem cells is mostly in neurodegenerative diseases, such as Parkinson's disease, partly in pre-clinical experiments [13], and in animal models of stroke, such as pigs and rats, transplantation of neural stem cells can improve neurological function and reduce disability [14];

Therefore, for subsequent transplantation treatment, how to obtain stable good cells, is also important, through our study, HUMSCs cultured in neural stem cell medium after adding hbFGF-2/N^2 for pre-induction treatment, the late differentiated neural stem cells, with good proliferation viability, improved the value-added rate of neural stem cells, and the induced differentiation of corresponding neural stem cells, in cell morphology, gene, protein and other levels of detection also have neural Stem cell marker gene and protein expression and morphological changes (e.g., Figures 2, 3 and 4) also showed significant expression differences compared to the strongly uninduced cells, and the differences were statistically significant; therefore, there was a stable source of neural stem cells through the method of early induction. The subsequent studies on how neural stem cells differentiate into the neural cells we need, how the microenvironment is regulated, how to move the damaged area, safety, etc., all need to be further investigated; the previous studies have a rat stroke model and a pig stroke model, whether the monkey stroke model, which is closer to human, can be used, and further validation is needed to provide some theoretical basis for further clinical experiments in the future. It also needs to be further validated to provide a theoretical basis for further clinical trials.

5. Conclusion

In conclusion, this study successfully differentiated and

cultured neural stem cells (NSCS) from human umbilical cord stromal cells (HSCS), and increased proliferation to obtain more neural stem cells, and a series of validations were performed by morphological, genetic and protein levels to provide an experimental basis for further experiments to follow.

Acknowledgements

This work was supported by the major frontier project of Sichuan Science and Technology Department Fund No.2018JY0016.

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