Neural differentiation of human Wharton's jelly-derived mesenchymal stem cells improves the recovery of neurological function after transplantation in ischemic stroke rats

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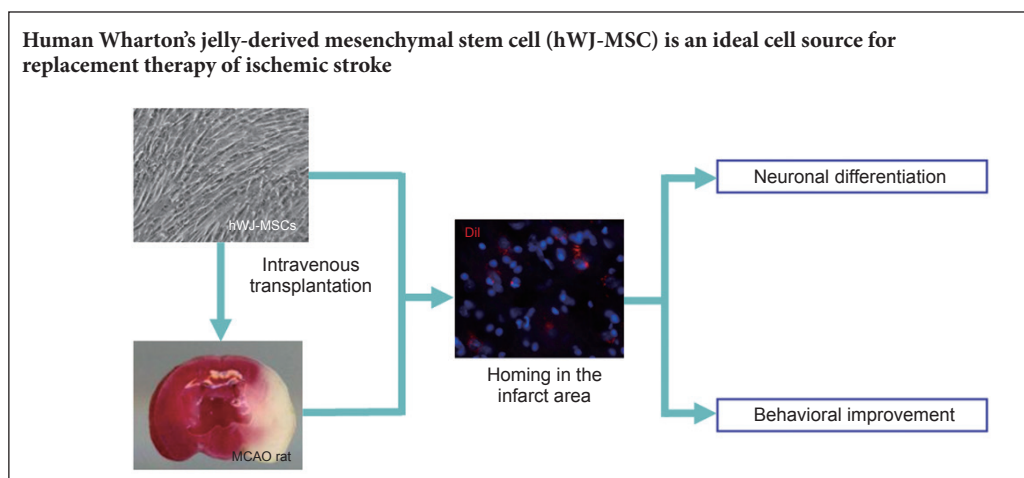4 Department of Obstetrics and Gynecology, the Affiliated Hosptial of Nantong University, Nantong, Jiangsu Province, China
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Graphical Abstract

Human Wharton's jelly-derived mesenchymal stem cell (hWJ-MSC) is an ideal cell source for replacement therapy of ischemic stroke

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Abstract

Human Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) have excellent proliferative ability, differentiation ability, low immunogenicity, and can be easily obtained. However, there are few studies on their application in the treatment of ischemic stroke, therefore their therapeutic effect requires further verification. In this study, hWJ-MSCs were transplanted into an ischemic stroke rat model *via* the tail vein 48 hours after transient middle cerebral artery occlusion. After 4 weeks, neurological functions of the rats implanted with hWJ-MSCs were significantly recovered. Furthermore, many hWJ-MSCs homed to the ischemic frontal cortex whereby they differentiated into neuron-like cells at this region. These results confirm that hWJ-MSCs transplanted into the ischemic stroke rat can differentiate into neuron-like cells to improve rat neurological function and behavior.

Key Words: nerve regeneration; human Wharton's jelly-derived mesenchymal stem cells; ischemic stroke; cell transplantation; middle cerebral artery occlusion; neural differentiation; neurological function; neural regeneration

Introduction

Ischemic stroke is a primary cause of death and long-term disability and is of huge social and economic burden worldwide (Zhao et al., 2014; Auer et al., 2015; Kawle et al., 2015). Following ischemic stroke, penumbra apoptosis and core necrosis in the infarct region can be seen within minutes to days (Deshpande et al., 1987; Leist and Jaattela, 2001; Du et al., 2014). Neuronal death following ischemia is strongly linked to the interruption of blood supply to brain regions in which nutrients and oxygen cannot be delivered as a result of thrombus occlusion (Cui et al., 2012).

There is increasing interest in the therapeutic potential of stem cell technology to treat acute ischemic stroke. Many studies report that a variety of cells, such as bone marrow-derived mesenchymal stem cells (BMSCs) and neural stem cells, can reduce brain damage induced by ischemia and promote the recovery of neurological function in animal models of middle cerebral artery occlusion (MCAO) (Dharmasaroja, 2009; Goldmacher et al., 2013; Jensen et al., 2013; Du et al., 2014; Tao et al., 2014; Yang et al., 2014; Cheng et al., 2015). It has been found that mesenchymal stem cells (MSCs) not only have the ability to renew, but can also differentiate into various cell lineages, including chondrocytes, osteocytes, and neurocytes, and possess "immunosuppressive" property. All of these characteristics make them an important candidate for allogeneic cell therapy (Pelizzo et al., 2015; Toyoshima et al., 2015; Xue et al., 2015). It is well known that bone marrow represents a major source of MSCs. However, due to the small cell number, decreasing proliferative capacity with age, and the high extent of viral infection, the use of BMSCs is not always acceptable (Rao and Mattson, 2001). In recent decades, researchers have found that MSCs also exist in human umbilical cord blood (Goodwin et al., 2001; Kakinuma et al., 2003). However, because of the low count of human MSCs per volume, umbilical cord blood is not an ideal source of MSCs for clinical use. Additionally, MSCs isolated from Wharton's jelly of the umbilical cord, termed human Wharton's jelly-derived MSCs (hWJ-MSCs), have also been identified. The superior characteristics of hWJ-MSCs, including excellent proliferative potential, the ability to differentiate into various cell lineages, low immunogenicity, and easy sample collection, make them a promising alternative cell type for basic research and clinical application for treatment of various diseases (Ali et al., 2015; Borhani-Haghighi et al., 2015; Li et al., 2015; Subramanian et al., 2015). Although evidence from Li et al. (2015) shows functional recovery in models of ischemia/reperfusion injury after hWJ-MSC transplantation, no studies have so far reported the homing and destiny of implanted hWJ-MSCs in the infarct region of the acute stroke brain.

In the present study, we cultured hWJ-MSCs *in vitro* and transplanted them into a rat MCAO-invoked stroke model. We evaluated their survival and differentiation *in vivo*, and their potential to improve behavioral deficiencies to provide evidence for using hWJ-MSCs as an ideal cell source for replacement therapy following ischemic stroke.

Materials and Methods

Animals

Forty-eight healthy adult female specific-pathogen-free Sprague-Dawley rats weighing 200–250 g and three pregnant Sprague-Dawley rats were provided by the Experimental Animal Center of Nantong University of China (license No. SYXK (Su) 2015-0031). All rats were caged in an approved animal facility with free access to food and water, and were kept in a temperature-controlled environment in a 12-hour light/dark cycle. All animal experiments were conducted in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The rats were randomly divided into sham group (sham operation; *n* = 12), saline group (MCAO + saline; *n* = 18) and transplantation group (MCAO + cell transplantation; *n* = 18).

Ischemic stroke model establishment by MCAO

The rat middle cerebral artery (MCA) was permanently occluded as a focal cerebral ischemia model with the modified Longa's method (Longa et al., 1989) (**Figure 1A**). Briefly, after rats were intraperitoneally anesthetized with chloropent anesthesia (2 mL/kg body weight), the surgical procedure was performed on a heated surface to avoid hypothermia. The right common carotid artery, external carotid artery and internal carotid artery were exposed. Silicon coated 4-0 monofilament with its tip rounded (Doccol, Redlands, CA, USA) was inserted *via* the external carotid artery into the internal carotid artery (**Figure 1A**), and then to the circle of Willis to occlude the origin of the right MCA. Two hours after MCAO, the nylon suture was withdrawn. The sham operation consisted only of a similar surgical procedure to expose the carotid artery, but without occlusion or injection.

After right MCAO, rats presented spontaneous leftwards turning because of left paralysis of limbs. Some rats did not show appropriate rotation and some failed to survive after the surgery. These rats were removed from the study. In total, 48 rats (approximately 64%) were obtained with successful MCAO.

2,3,5-Triphenyltetrazolium chloride (TTC) staining

At 24 hours after surgery, the rats were decollated and the brain was rapidly removed. After being frozen for 10 minutes at –20°C, brains were sectioned into 2 mm-thick slices using a vibratome, immersed for 15 minutes in 1.5% TTC solution at 4°C, then subjected to histological analysis and observed using a phase contrast microscope (Leica, Heidelberg, Germany).

Isolation of hWJ-MSCs

Preparation of hWJ-MSCs was approved by the Research Ethics Committee at the Affiliated Hospital of Nantong University of China. Informed consent was obtained from all pregnant women and their family members. Cells were isolated and cultured at Beike Biotechnology (Taizhou, Jiangsu Province, China). Fresh human umbilical cords were obtained from the Affiliated Hospital of Nantong University of China, and stored in iced Hanks' balanced salt solution

(Gibco, Grand Island, NY, USA) at 4°C and processed within 2 hours after birth. After being rinsed in 75% ethanol for 30 seconds, umbilical cords were cut into segments (2–3 cm long). Afterwards, the umbilical cord arteries and veins were gently removed to avoid contamination with endothelial cells. The mesenchymal tissue (Wharton's jelly) was dissected into small pieces of approximately 0.5 cm^3 and transferred into a flask containing StemPro® MSC serum-free medium (Life Technologies, Invitrogen, Carlsbad, CA, USA) with antibiotics (penicillin 100 IU/mL, streptomycin 100 μg/mL; Invitrogen) and 10% fetal bovine serum (Invitrogen). The explants were cultured in a humidified 95% air 5% (v/v) CO_2 incubator at 37°C for 3–4 days without disturbance to allow migration from the explants. Following cell migration from the explants, the tissue masses were removed, and the media were half replaced by fresh media twice weekly. After approximately 2 weeks, the cells reached 90–100% confluence and were passaged. Cells at passage 3 were used experimentally or stored in liquid nitrogen for further use.

Identification of hWJ-MSCs

hWJ-MSCs at passage 3 were harvested and stained with the following antibodies: phycoerythrin-conjugated mouse anti-human CD34, CD73, CD105, and HLA-DR; allophycocyanin-conjugated mouse anti-human CD79a and CD90 (BD Pharmingen, San Diego, CA, USA). The isotype-matched immunoglobulins IgG1-phycoerythrin and IgG1-allophycocyanin were used as negative controls under the same conditions. All steps were performed according to the manufacturer's instructions. The profiles of hWJ-MSCs were analyzed by flow cytometry (FACSCalibur, Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Identification of adipogenic and osteogenic differentiation

hWJ-MSCs at passage 3 were used to detect adipogenic and osteogenic differentiation potential using different media. Adipogenic differentiation medium contained 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 10 μg/mL insulin, 100 μM indomethacin, and 10% fetal bovine serum in DMEM/F12. Osteogenic differentiation medium contained 0.1 μM dexamethasone solution, 0.2 mM ascorbic acid 2-phosphate solution, 10 mM glycerol 2-phosphate, and 10% fetal bovine serum in DMEM/F12. Cells at 100% confluence were incubated in these different media. Two weeks later, the cells were incubated with 0.375% oil red O (Sigma, St. Louis, MO, USA) or 0.5% Alizarin red S (Sigma) for 30 minutes to identify their potentiality to differentiate into adipocytes and osteoblasts, respectively. The oil red O and Alizarin red S were removed and imaging was performed after the cells were air-dried.

Transplantation of hWJ-MSCs

hWJ-MSCs at passage 3 were harvested and incubated with Cell Tracker CM-Dil (3 μM; Invitrogen) for 5 minutes at 37°C, and an additional 15 minutes at 4°C. The cells were washed in phosphate buffered saline and filtered through a 100-μm filter. Cells were resuspended in saline and placed in an ice bath before cell transplantation. Subsequently, approximately 1×10^7 cells in 200 μL cell suspension were injected into the MCAO model rat through the tail vein 2 days after surgery (**Figure 2A**). An identical volume (200 μL) of saline was given to the saline group through the tail vein 2 days after surgery.

Behavioral studies

Functional behavior following transplantation was monitored in all groups as the schedule shown in **Figure 2A**. All behavioral tests were performed by an experimenter who was blinded to the experimental protocol.

Longa scoring

According to the 5-grade scoring standard of Longa et al. (1989), all rats were evaluated at 6, 72 hours, 7, 20 and 30 days after cell transplantation. The scoring criteria were as follows: 0 = Normal, no neurological function defect; 1 = forelimb flexion; 2 = unidirectional circling; 3 = falling to the contralateral side; 4 = decreased level or lack of consciousness.

Rotarod test

The rats were trained for 3 consecutive days at 20 days after cell transplantation. According to a previous method (Goel et al., 2016), rats were placed on an accelerating rotarod cylinder and the speed was slowly increased from 4 to 40 r/min within 4 minutes. The longest time of three independent measurements that rats remained on the rotarod (rotarod latency) was recorded.

Morris water maze test

The learning and memory abilities of the rats were assessed using the Morris water maze test. The Morris water maze test (de Bruin et al., 1997) was performed at 30 days after cell transplantation. The rats learned to locate a circular platform at a fixed location every day (5 trials per day). In each trial, the rat was placed into the water at one of three designated start-points on the wall of the tank. Escape latency (time to find the platform) and time in each quadrant were measured using an auto-tracking system. If rats were unable to find the platform within 120 seconds, the escape latency was recorded as 120 seconds. The average escape latency and time in each quadrant of five tests per day was used for statistical analysis.

Immunofluorescence staining

At 35 days after hWJ-MSCs transplantation, the rats were sacrificed and the brains were collected and fixed in 4% formalin. The coronal sections (15 μm thickness) through the area of ischemia were prepared using a cryostat (CM1900; Leica, Heidelberg, Germany). The sections were blocked in 10% goat serum in phosphate-buffered saline/Tween (0.01 M sodium phosphate buffer, pH 7.4, 0.05% Tween 20) for 1 hour at room temperature, and incubated with the primary antibody diluted in blocking buffer at 4°C for 24 hours, followed by incubation with secondary antibody overnight at 4°C. Immunofluorescence signals were observed under a fluorescence microscope (DMIRB; Leica). Primary antibodies were as follows: guinea pig anti-doublecortin (1:1,000; Millipore, Boston, MA, USA), mouse anti-microtubule-associated protein 2 (MAP2) (1:1,000; Millipore) or mouse anti-Tuj1 (1:400; Sigma). Secondary antibodies were as follows:
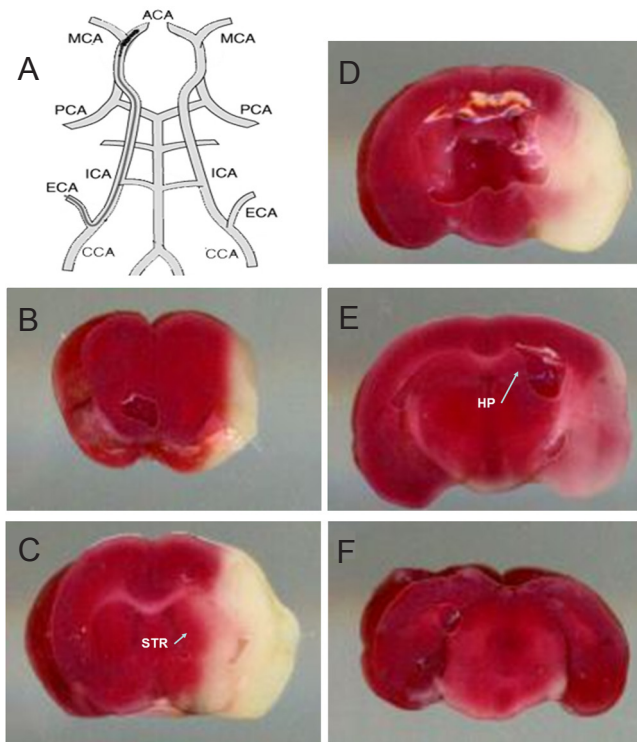
Figure 1 Model establishment and confirmation of ischemic stroke.
(A) Diagram of MCAO: Formation of branches from the carotid artery. (B–F) Anteroposterior brain coronal sections stained with 2,3,5-triphenyltetrazolium chloride at 24 hours after MCAO. The pale and conical area indicates the infarct tissue. CCA: Common carotid artery; ECA: external carotid artery; ICA: internal carotid artery; MCA: middle cerebral artery; PCA: posterior cerebral artery; ACA: anterior cerebral artery; HP: hippocampus; STR: striatum; MCAO: middle cerebral artery occlusion.

Alexa fluor 488-conjugated goat anti-guinea pig IgG (1:1,000; Invitrogen, Carlsbad, CA, USA) and FITC-conjugated goat anti-mouse IgG (1:1,000; Millipore). After that, sections were counterstained with Hoechst (1:1,000) to indicate cell nuclei.

Statistical analysis
Statistical analysis was performed using GraphPad Prism v4.0 software (GraphPad Software, San Diego, CA, USA). Data are expressed as the mean ± standard error of the mean (SEM). The differences between groups were analyzed using the unpaired *t*-test. $P < 0.05$ was considered statistically significant.

Results
Infarct area in rat models of ischemic stroke
At 24 hours after surgery, TTC results showed that the range of infract area (pale) on cortical surface was from bregma 1.70 mm to bregma −6.30 mm. The whole ischemic area size in the surgical side was conical in shape with the base of the cone facing the cortex. The most serious area of ischemia was in the frontal cortex, while the striatum and hippocampus were barely affected (**Figure 1B–F**).

Culture and identification of hWJ-MSCs
On day 4 after primary culture, cells grew as a monolayer and exhibited a shape with a flat and polygonal morphology (**Figure 3A**). When cells at passage 3 reached approximately
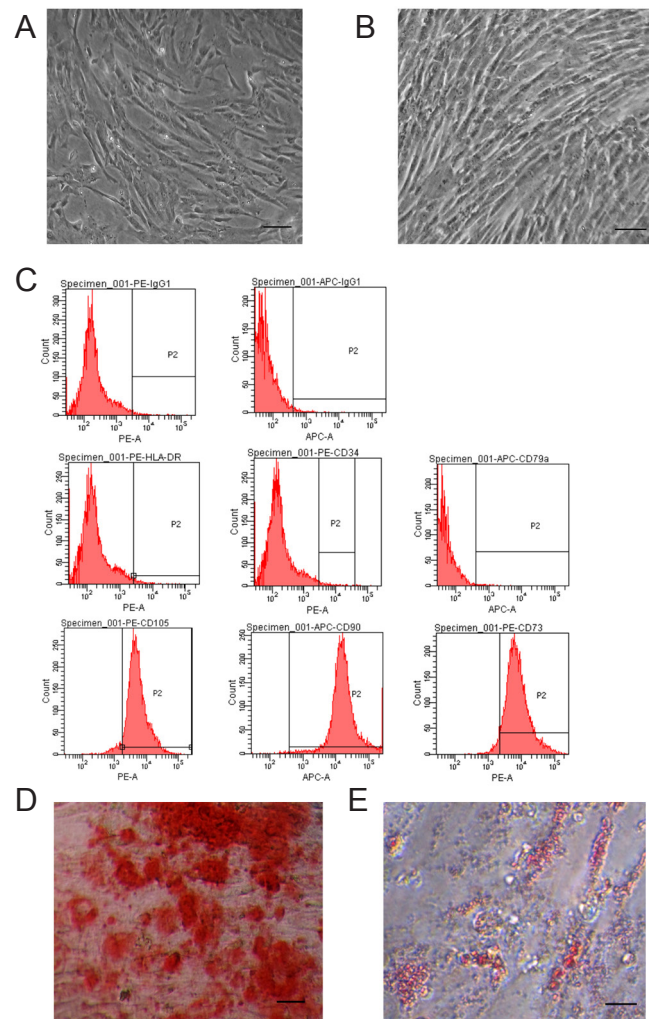

Figure 3 Identification of mesenchymal stem cells from human Wharton's jelly *in vitro*.
(A) Culture in passage 1 showing some cell migration from the edge of the tissue pieces and a flat or polygonal morphology. (B) Culture in passage 3 showing most cells presenting as spindle-shaped and on reaching approximately 90% confluence arranged in a parallel or whirlpool pattern. (C) Identification of cell phenotype by flow cytometric analysis. Results show that cells were positive for CD73, CD90, and CD105, but negative for CD34, CD79a, and HLA-DR. (D, E) Induction of adipogenic and osteogenic differentiation. After being cultured with standard osteogenic and adipogenic differentiation media, most cells were positive for Alizarin red S (D) and Oil Red O staining (E), respectively. Scale bars: 50 μm.

90% confluence, they mostly presented spindle-shaped and were arranged in a whirlpool pattern (**Figure 3B**). Flow cytometric analysis demonstrated that the third passage cells were positive for MSC markers CD73, CD90, and CD105, and negative for the hematopoietic and endothelial markers CD34, CD79a, and HLA-DR (**Figure 3C**).

To investigate multipotential differentiation of the cultured cells, osteogenic and adipogenic differentiation experiments were carried out. Following treatment with standard osteogenic and adipogenic differentiation media, most adherent cells were positive for Alizarin red S (**Figure 3D**) and Oil red O (**Figure 3E**) staining, respectively. These results demonstrated that the cells had the phenotype and differentiation characteristics of MSCs.
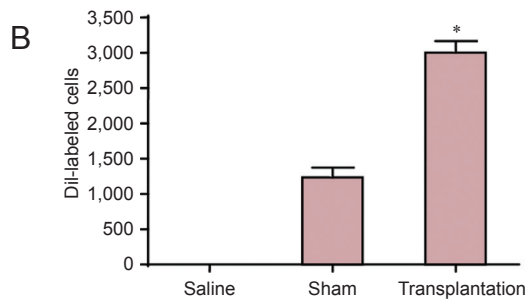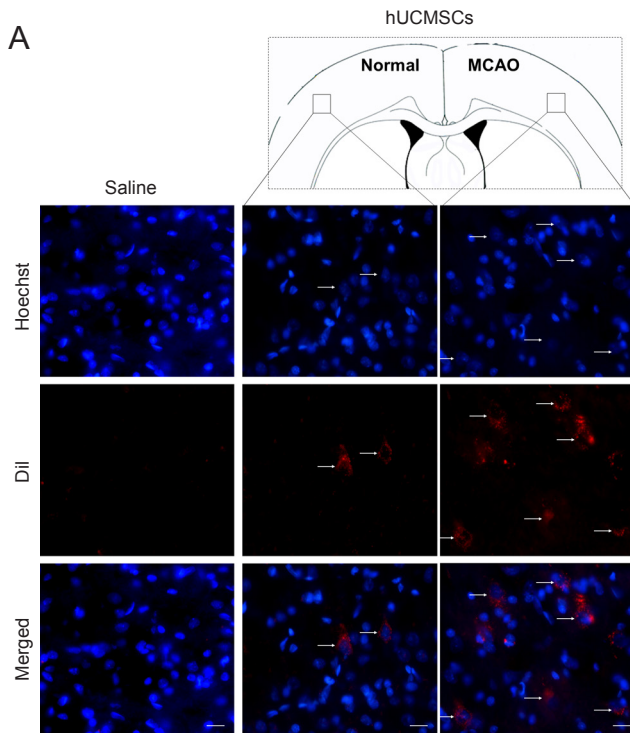
Figure 4 Transplanted hWJ-MSCs in the infarct and corresponding areas of the ischemic stroke model rat.
(A) Distribution of transplanted hWJ-MSCs (immunofluorescence staining). Typical photographs of the CM-Dil-labeled cells (red, arrows) in the infarct or normal area of MCAO rats with or without injection of hWJ-MSCs. Hoechst is blue. Scale bars: 25 μm. (B) Quantification of the number of CM-Dil-labeled cells in the normal and MCAO sides of the frontal cortex between bregma 1.70 mm and 0.20 mm. Data are expressed as the mean ± SEM and analyzed by unpaired *t*-test. *$P < 0.05$, *vs*. sham group ($n = 6$). MCAO: Middle cerebral artery occlusion; hWJ-MSCs: human Wharton's jelly-derived mesenchymal stem cells.
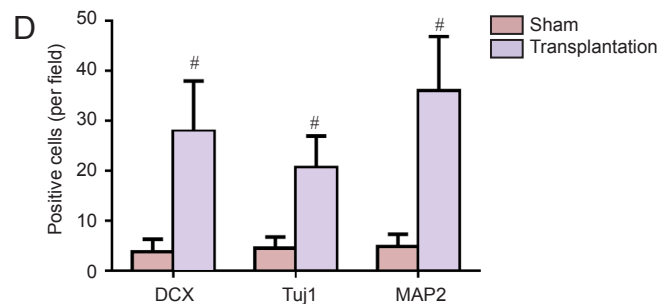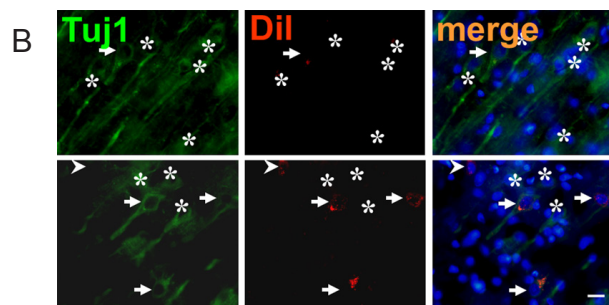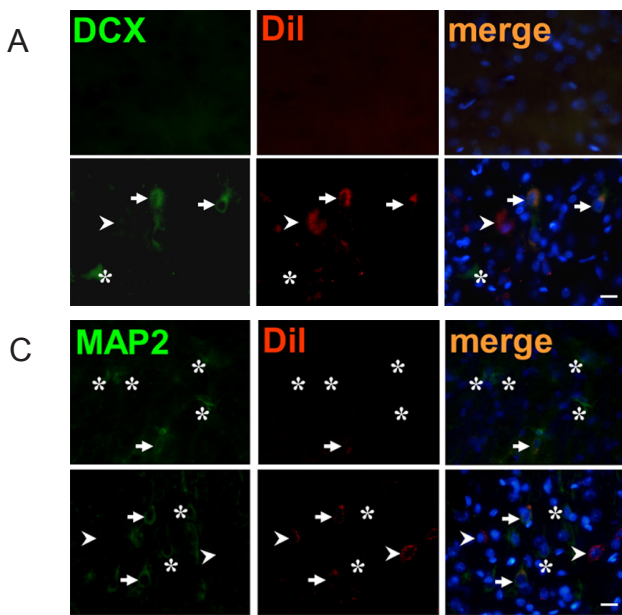
Figure 5 Detection of neuronal differentiation of the transplanted CM-Dil-labeled hWJ-MSCs in the cortex of the ischemic stroke model rat.
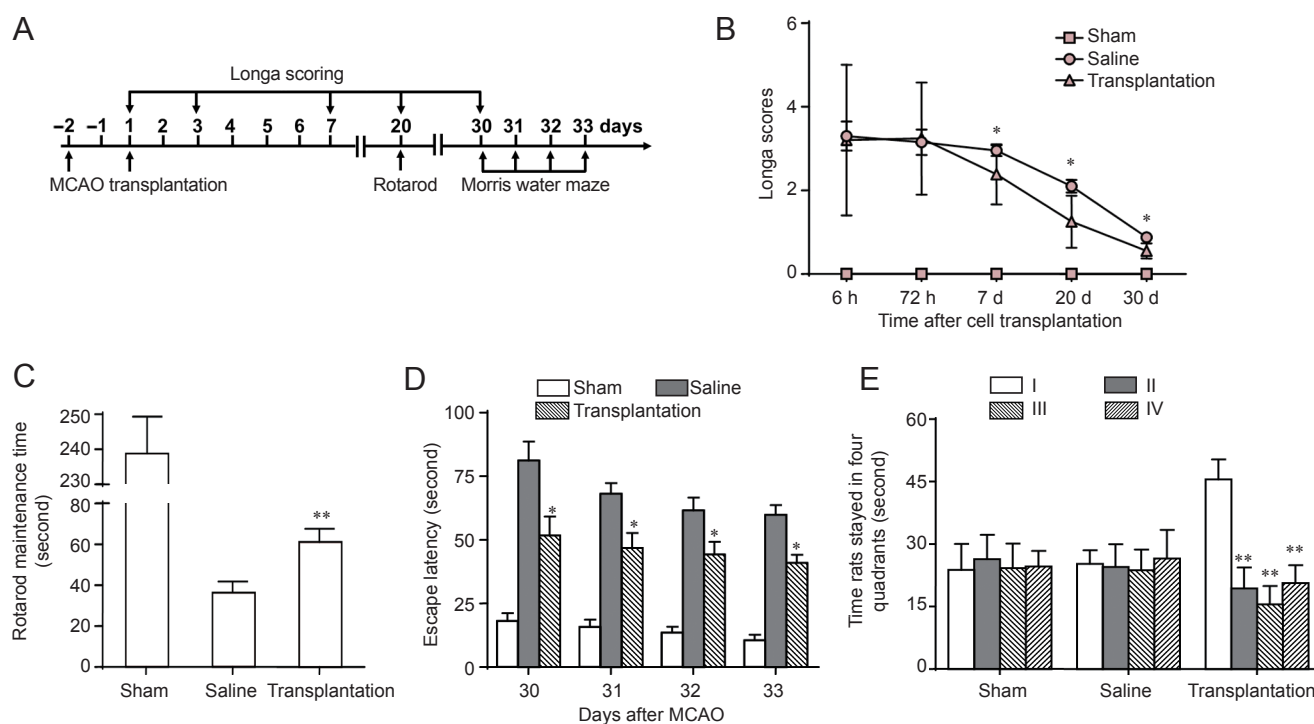At 35 days after cell transplantation, coronal sections crossing the infarct (lower) and corresponding areas (upper) were immunostained with anti-DCX (A), Tuj1 (B) and MAP2 (C) antibodies. DCX, Tuj1 and MAP2 are all green, CM-Dil is red and Hoechst is blue. Arrows indicate DCX-, Tuj1- or MAP2-positive CM-Dil-labeled cells. Arrowheads indicate DCX-, Tuj1- or MAP2-negative CM-Dil-labeled cells. Asterisks indicate the DCX-, Tuj1- or MAP2-positive, but not CM-Dil-labeled cells ($n = 6$). Scale bars: 25 μm. (D) Quantification of DCX-, Tuj1- or MAP2-positive CM-Dil-labeled cells in a 20-fold field in A, B and C. Data are expressed as the mean ± SEM and analyzed by the unpaired *t*-test. #$P < 0.05$, *vs*. normal side ($n = 6$). DCX: Doublecortin; MAP2: microtubule-associated protein 2; hWJ-MSCs: human Wharton's jelly-derived mesenchymal stem cells.

Behavioral improvement of the ischemic stroke model rat after hWJ-MSC transplantation
Behavioral tests were performed timely as exhibited in **Figure 2A**. Longa scoring results showed that there was no significant difference between the transplantation group and the saline group at 6 and 72 hours after cell transplantation.

However, at 7, 20 and 30 days after transplantation, the Longa scores of rats in the transplantation group were significantly lower than those in the saline group at the corresponding time points ($P < 0.05$), although they did not reach the levels of the sham rats ($P > 0.05$) (**Figure 2B**).

In the rotarod test, rats were trained for 3 consecutive days

Figure 2 Behavioral improvement of MCAO rats after hWJ-MSC transplantation.
(A) Schedule of surgery, cell transplantation and behavior test. (B) Longa scoring was performed at 6, 72 hours, 7, 20 and 30 days after cell transplantation. (C) Maintenance time in the rotarod test, which was carried out on day 20 after cell transplantation. (D, E) Escape latency (D) and time the rats stayed in four quadrants (I–IV, E) in the Morris water maze, which was performed continually for 4 days on day 30 after cell transplantation. Data are expressed as the mean ± SEM and analyzed by unpaired *t*-test. *$P < 0.05$, **$P < 0.01$, *vs.* saline group. MCAO: Middle cerebral artery occlusion; hWJ-MSCs: human Wharton's jelly-derived mesenchymal stem cells.

before MCAO and evaluated at 20 days after cell transplantation. Results showed that the rotarod latency in the transplantation group was longer than that in the saline group ($P < 0.01$; **Figure 2C**).

The Morris water maze test results indicated that rats transplanted with hWJ-MSCs showed a decrease in escape latency compared with the saline group (**Figure 2D**) and the time they stayed in the target quadrant also increased significantly ($P < 0.01$; **Figure 2E**).

Transplanted hWJ-MSC location and survival in the infarct area of the ischemic stroke model rat
To verify whether transplanted hWJ-MSCs could reach the infarct area and survive, we performed a tracing experiment. The transplanted hWJ-MSCs were labeled with CM-Dil before tail vein injection. Immunofluorescence staining results showed that the number of hWJ-MSCs in the infarct area was greater than that in the corresponding area of the controlateral side in the transplantation group ($P < 0.01$). In the saline group, there were no hWJ-MSCs detected on the MCAO side (**Figure 4**).

***In vivo* neuronal differentiation of transplanted hWJ-MSCs in the ischemic stroke model rat**
At 35 days post-transplantation, immunofluorescence staining results showed that approximately 25.17 ± 1.2%, 18.13 ± 0.57% and 12.36 ± 1.39% of the implanted cells expressed the neuronal markers doublecortin, Tuj1 and MAP2 (Ng et al.,

2012; Castaño et al., 2014), respectively, in the infarct area (**Figure 5A–C**). Quantification indicated that the numbers of doublecortin-, Tuj1- and MAP2-positive cells labeled with CM-Dil were greater in the MCAO region compared with the normal brain ($P < 0.05$) (**Figure 5D**). However, neuronal differentiation was seldom observed in the corresponding area on the normal side.

Discussion
The MSC is an important member of the stem cell family, characterized by strong self-renewal and multi-differentiation potentials. Romanov et al. (2003) successfully isolated and cultured human MSCs from umbilical cord vasculature and Wharton's jelly, and verified that these cells contain MSC-like properties, so named them hWJ-MSCs. Since then, more studies have indicated that hWJ-MSCs can differentiate into neurons under specific conditions (Mitchell et al., 2003; Balasubramanian et al., 2013). hWJ-MSCs are in abundant supply and easy to obtain with no ethical limits. Furthermore, hWJ-MSCs possess almost all characteristics of MSCs and have minimal immunogenicity, which is beneficial for their long-term survival in the host brain. Therefore, hWJ-MSCs are expected to become a promising source for treatment of neurodegenerative diseases (Porada et al., 2006; Noël et al., 2007).

In this study, hWJ-MSCs were isolated from Wharton's jelly of human umbilical cord and expanded *in vitro*. Flow cytometric analysis demonstrated that these cells had char-

acteristics of MSCs, with positive expression of the markers CD105, CD73 and CD90, and negative expression of CD34, HLA-DR and CD79a. CD105, CD73 and CD90 are not 'specific' to MSCs, but their expression profile helps to identify them. Intravenous transplantation was deemed to be a suitable method (Chen et al., 2001; Doeppner and Hermann, 2014; Zhang et al., 2014). In this study, the cells were transplanted into rat MCAO models *via* the tail vein. A comparative study indicates that the CM-DiI cell tracker is much less diffuse than other standard DiI analogues (Daubeuf et al., 2009), and was therefore used to label the cells prior to transplantation (Qiao et al., 2015). We observed that hWJ-MSC transplantation significantly improved the neurological function of MCAO rats compared with the saline control at 7 days after transplantation. Furthermore, we found more implanted hWJ-MSCs in the infarct region than the normal side, and some cells in the infarct area differentiated into neurons at 35 days after transplantation. Furthermore, neurological damages in behavior were also partially alleviated. These results indicate that exogenous hWJ-MSCs injected *via* the tail vein can migrate into the infarct area of the MCAO rat, survive and even differentiate into neurons to partially rescue the damaged motor function. However, the mechanisms by which the implanted hWJ-MSCs improved neurological behavior of the MCAO models remain unclear. Some researchers consider that MSCs transplanted into the MCAO rat can differentiate into mature neurons, which can form a local neural circuit with the host nervous cells and replace the damaged neurons to some extent (Kim et al., 2008). Other researchers speculate that the transplanted MSCs promote endogenous neural stem cell proliferation (Yoo et al., 2008), which is partially responsible for the recovery of neurological function. Xin et al. (2013) reported that after injecting MSCs through the tail vein, expression of transforming growth factor β-1 decreased in microglia and macrophages of MCAO rats and the inhibitor of plasminogen activator was also reduced, resulting in the activation of plasminogen activator and matrix metalloproteinase (Adibhatla and Hatcher, 2008). Subsequently, proliferation of astrocytes was reduced, and migration and neurite extension of neurons was promoted (Hosomi et al., 2001). Therefore, the effects on the regulation of glial cells may play an important role in the treatment of MCAO rats with hWJ-MSC transplantation. In addition, we observed that the neurological function of MCAO rats in the saline group could be partially recovered with prolonged time.

Orito et al. (2010) reported that cerebrospinal fluid extracted from rats 15 minutes after MCAO could promote proliferation of BMSCs *in vitro*. Yang et al. (2010) injected BMSCs into MCAO rats through the tail vein at 1 day after surgery and found that some factors, such as interleukin 13, vascular endothelial growth factor and nerve growth factor receptor, tended to be up-regulated and efficiently promoted the recovery of neurological function. All of these studies indicate that in a certain period of time after MCAO, the internal environment of rats and the exogenous MSCs can form a complementary relationship. In the present study, we found that the transplanted hWJ-MSCs were more dynamic in migration into the infarct area than to the corresponding area on the normal side. This prompted that MCAO injury may stimulate the model animal to secrete factors to benefit migration, survival and proliferation of the transplanted hWJ-MSCs, and that these cells may continuously stimulate the host to secrete factors for the improvement of neurological function. The factors need to be studied further in future.

In summary, exogenous hWJ-MSCs transplanted into MCAO rats through tail vein injection can locate and survive in the infarct area. Furthermore, some of these cells differentiate into mature neurons and lead to significant recovery of the function of the MCAO rats. These findings suggest that hWJ-MSC transplantation has significant potential for clinical application in the treatment of ischemic stroke.

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