Human umbilical cord-derived mesenchymal stem cells promote repair of neonatal brain injury caused by hypoxia/ischemia in rats

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Abstract

Administration of umbilical cord-derived mesenchymal stem cells (UC-MSCs) is believed to be an effective method for treating neurodevelopmental disorders. In this study, we investigated the possibility of UC-MSCs treatment of neonatal hypoxic/ischemic brain injury associated with maternal immune activation and the underlying mechanism. We established neonatal rat models of hypoxic/ischemic brain injury by exposing pregnant rats to lipopolysaccharide on day 16 or 17 of pregnancy. Rat offspring were intranasally administered UC-MSCs on postnatal day 14. We found that polypyrimidine tract-binding protein-1 (PTBP-1) participated in the regulation of lipopolysaccharide-induced maternal immune activation, which led to neonatal hypoxic/ischemic brain injury. Intranasal delivery of UC-MSCs inhibited PTBP-1 expression, alleviated neonatal brain injury-related inflammation, and regulated the number and function of glial fibrillary acidic protein-positive astrocytes, thereby promoting plastic regeneration of neurons and improving brain function. These findings suggest that UC-MSCs can effectively promote the repair of neonatal hypoxic/ischemic brain injury related to maternal immune activation through inhibition of PTBP-1 expression and astrocyte activation.

Key Words: developmental brain disease model; disease-associated astrocytes; intranasal administration; lipopolysaccharide; maternal immune activation; neonatal brain injury; neuroplasticity repair; polypyrimidine tract-binding protein-1; stem cell therapy; umbilical cord-derived mesenchymal stem cells

Introduction

Neonatal brain injury (NBI) is generally induced by hypoxia/ischemia, inflammation, or infection during critical periods of development. NBI can lead to long-term neurodevelopmental abnormalities, including motor impairments, mental and developmental retardation, learning disabilities, and psychiatric disorders (Li et al., 2017a; Pierrat et al., 2017). Clinical studies have shown that children born to mothers exposed to viral and bacterial pathogens during pregnancy are at higher risk of developing various neurological diseases, including cerebral palsy, schizophrenia, autism, bipolar disorder, major depression, and epilepsy (Burd et al., 2012; Mwaniki et al., 2012; Bergdolt and Dunaevsky, 2019). Experimental models have shown that lipopolysaccharide (LPS)-induced prenatal maternal immune activation (MIA) can lead to activation of astrocytes and microglia and to significant declines in the long-term neuronal plasticity of offspring (Canetta et al., 2016; Estes and McAllister, 2016).

In newborns, current research suggests that neuronal circuits involved in fine motor control, motor learning, and cognitive function are particularly susceptible to damage from systemic inflammation and hypoxia (Frangopoulou et al., 2019). Studies have consistently demonstrated that disease-associated astrocytes (DAAs) respond to inflammatory damage by promoting release of inflammatory factors, which accelerate activation of astrocytes and microglia (Chávez et al., 2019; Wang et al., 2019; Hampel et al., 2020). DAAs stimulate neuronal repair and regeneration by inducing transdifferentiation and by inhibiting release of proinflammatory cytokines (Qian et al., 2020). Astrocytes exhibit different activation states depending on functional localization, which is distinguishable by the expression levels of glial fibrillary acidic protein (GFAP) (Habib et al., 2020). Reactive astrocytes can be categorized into A1 or A2 subtypes (Vismara et al., 2020), with A1 “harmful” astrocytes leading to aggravation of brain injury and A2 “beneficial” astrocytes playing a protective role in neurons (Chen et al., 2019). Expression of A1 reactive astrocytes, or DAAs, has been shown to be induced by classically activated...
neuroinflammatory microglia and can be identified by higher expression levels of GFAP (Liddelow et al., 2017; Habib et al., 2020).

After brain injury, activated astrocytes have been shown to undergo in situ conversion into neurons through a process called transdifferentiation, with poly-pyrimidine tract-binding protein-1 (PTBP-1) playing a key role in the process (García-Blanco et al., 1989). Recent research has suggested that these transdifferentiated cells can replace lost neurons and reconstitute endogenous neural circuits (Gascón et al., 2017). PTBP-1, which is a system-dependent transcription factor with in vivo reprogramming effects, may therefore be a key factor and therapeutic target in neurological diseases. Previous studies have confirmed PTBP-1’s role in a murine model of degenerative brain diseases; however, this protein has never before been studied in a developmental model of NBI (Liu et al., 2020; Qian et al., 2020; Zhou et al., 2020).

Human umbilical cord mesenchymal stem cell (UC-MSC) transplantaion has been shown to be a powerful method for stimulating endogenous repair processes, leading to changes in behavioral outcomes and volumes of cerebral lesion after neonatal brain damage. Mesenchymal stem cells (MSCs) can inhibit astrocyte activation, reduce expression of inflammatory factors, and replace lost neurons (Thomi et al., 2019a; Xu et al., 2019). New developments in intranasal administration and exosome application of stem cells or their products are currently being explored in this therapy even safer and more precise (Drommelschmidt et al., 2019). Although the therapeutic effects of MSC transplantation have previously been reported (Dabrowska et al., 2019), methods for achieving sustained therapeutic efficacy have not been fully developed yet.

Our study investigated whether MIA-induced NBI is regulated by activation of PTBP-1 and how its expression in the attempt to find a therapeutic approach that would reverse the injury process and achieve neuroplasticity repair, thereby providing evidence for investigating the mechanism of stem cell treatment.

Materials and Methods

Animals and experimental design

Four pregnant Sprague-Dawley rats (gestational days [G] 16 and 17) were obtained from the specific-pathogen-free Experimental Animal Center of Dalian Medical University (Dalian, China; SCKER2018-0003) and housed individually in standard plastic cages (Liaoning Normal University). The rats were allowed free access to food and water under controlled temperature and a 12-hour light/dark cycle. The four pregnant rats were randomly assigned to two groups: two were injected intraperitoneally with 0.5 mg/kg LPS (Escherichia coli O111:B4, L2630-10 mg, Sigma-Aldrich, Shanghai, China; LPS-normal saline = 1:1) and the other two were injected intraperitoneally with 0.5 mg/kg PBS on G16 and G17. In the control group, 13 pups (male:female = 6:5) were delivered. On postnatal day 1 (P1), the male and female pups participating in the study were numbered separately and randomly assigned to an individual feedingudy to the study groups. The randomization sequence was created using Stata 9.0 (Stata Corp., College Station, TX, USA) statistical software, which was center-stratified with a 1:1 allocation using random block sizes, and the grouping results were revealed only after the pups were killed and developmental stages assessed. The pups were assessed. Ear standing: the rats’ ears can stand up completely. If the ear edge is not attached to the coat, the result is set as positive. Righting reflex: if the young rat can successfully complete a turnover three times in a row within 20 seconds, the result is set as positive within 3 seconds. Incisor odontogenesis: if the operator’s finger touches the anterior teeth, it is positive. Auditory astonishment: a tuning fork is tapped with a predetermined force 10 cm from the young rat’s head, and then 1 × 10^5 µL were injected into each nasal cavity (5 µL were delivered intranasally to each rat of the treatment group) using FV10-ASW 3.1 software, and the purity of neurons was measured by fluorescence-activated cell sorting (FACS) analyses were performed using a Flow Cytometer (BD LSRFortessa, BD Biosciences).

Tissue preparation

On P40, pups were deeply anesthetized by an intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg). For western blot analysis, after rapidly removing the brain, the areas of interest were dissected and stored using a 3°C. For hematoxylin and eosin staining, Nissl staining, immunohistochemistry, and immunofluorescence, pup brains were perfusion-fixed with 4% paraformaldehyde, dehydrated with a 30% sucrose solution, and embedded in optimum cutting temperature compound for frozen sectioning, with a slice thickness of 4 µm. Starting from the same part of the brain tissue in each group, each slice of every 10 brain slices was selected for comparison to avoid subjective underestimation of the number of positive cells.

Hematoxylin and eosin and Nissl staining

Slices were washed with distilled water, stained with hematoxylin for 5 minutes, and then stained with eosin for 5 minutes. Alcohol, xylene, and neutral adhesives were used for dehydration, transparency, and sealing, respectively. Sections were removed from 4°C and left at room temperature for 30 minutes. They were then washed with double distilled water and a filtered tar violet solution was added for 2 hours. The sections were then rinsed and placed in xylene for 5 minutes each. The sections were then placed in a 100% alcohol solution followed by xylene for 5 minutes each. Sections were then taken out to dry and sealed with a neutral resin, after which the neutral gum was completely air-dried and observed under a light microscope.

Immunohistochemistry and immunofluorescence staining

Immunohistochemical staining

Brain tissue was fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 4-µm sections. The sections were deparaffinized in xylene, and then rehydrated using an alcohol gradient. Next, the sections were incubated overnight with an anti-PTBP-1 antibody (1:150, rabbit, Abcam, Cambridge, UK, Cat# ab133734) at 4°C, and then incubated with a secondary antibody (1:300, Goat anti-rabbit, Thermo Fisher Scientific, Fremont, CA, Cat# TL-125-QHL) at 25 ± 1°C for 30 minutes, followed by incubation with a biotin-diamino benzidine tetrachloride solution at 25 ± 1°C for 1 minute and counterstaining with Mayer’s hematoxylin. Images were captured using an optical microscope (Olympus).

Immunofluorescence staining

Frozen brain tissue sections were fixed by incubation with cold acetone for 30 minutes, and then permeabilized by incubation with 0.5% Triton X-100 in PBS for 10 minutes at 25 ± 1°C. Tissue sections were incubated overnight at 4°C with different primary antibodies, including anti-interleukin (IL)-1β (1:150, rabbit, Abcam, Cat# ab525460) and anti-IL-6 (1:200, mouse, Abcam Cat# ab102737), RRID: AB_11153119, mouse anti-human CD45-FITC (BD Pharmingen, Cat# 555859, RRID: AB_396146), mouse anti-human HLA-DR-FITC (BD Pharmingen, Cat# 555842, RRID: AB_395858) for 25 minutes at 4°C. After two washes, the cells were resuspended in PBS, Fluorescence-activated cell sorting (FACS) analyses were performed using a Flow Cytometer (BD LSRFortessa, BD Biosciences).

Isolation and identification of UC-MSCs

Umbilical cord specimens were provided by the Department of Obstetrics, The First Affiliated Hospital of Dalian Medical University. These specimens were taken from healthy fetuses delivered by cesarean section. Pregnant women and their family members signed written informed consent forms. The use of human samples was approved by the Ethics Committee of The First Affiliated Hospital of Dalian Medical University (No. LCRY2016-59) on November 16, 2016. Human UC-MSCs were extracted using the tissue block adherence method (Dominici et al., 2006). Within 24 hours, the umbilical cord, which was stored under aseptic conditions at 4°C, was cleaned and cut into 1–3 mm² segments in PBS containing 1% tri-antibody (C0223, 100 mL, Beyotime, Shanghai, China), and then soaked in a special medium of amniotic mesenchymal stem cells (Beijing Tongli Haoyuan Biotechnology Co., Ltd., Beijing, China). When the aqueous layer was placed on a 10-cm-diameter dish for 2 hours, 10 mL of culture medium was added, and the dish was placed in an incubator containing 5% CO₂ at 37°C. The medium was changed every 3 days. After the cells adhered, passage five times, when they reached 80–90% confluence, they were used for flow cytometry.

Single cell suspensions of UC-MSCs in PBS were incubated with mouse anti-human CD73-BV421 (BD Pharmingen, Franklin Lakes, NJ, USA, Cat# 562430, RRID: AB_11153119), mouse anti-human CD105-APC (BD Pharmingen, Cat# 562408, RRID: AB_11154045), mouse anti-human CD90-fluorescein isothiocyanate (FITC) (BD Pharmingen, Cat# 555859, RRID: AB_395961), mouse anti-human HLA-DR-FITC (BD Pharmingen, Cat# 555812, RRID: AB_396146), mouse anti-human CD34-PerCP-Cy5.5 (BD Pharmingen, Cat# 347203, RRID: AB_400266), or mouse anti-human CD45-FITC (BD Pharmingen, Cat# 555482, RRID: AB_395858) for 25 minutes at 4°C. After two washes, the cells were resuspended in PBS, Fluorescence-activated cell sorting (FACS) analyses were performed using a Flow Cytometer (BD LSRFortessa, BD Biosciences).
All 13 rats in the control group survived, whereas one of the 12 rats in the MIA-related NBI affects the ontogeny and brain morphology of neonatal rats. DR, CD34, and CD45 showed expression rates of 0.13%, 0.36%, and 0.18%, were expressed at 99.94%, 98.62%, and 99.86%, respectively, whereas HLA-DR, CD34, or CD45 analysis. After incubation with CD73, CD105, CD90, HLA-DR, CD34, or CD45 UC-MSCs identification the mean ± standard error of the mean (SEM) and were analyzed using SPSS Statistical analysis No statistical methods were used to predetermine sample sizes; however, our sample sizes were similar to those reported in previous publications (Jiao et al., 2019, 2021). No animals or data points were excluded from the analysis (the evaluator was blind to the grouping results). The data are presented as the mean ± standard error of the mean (SEM) and were analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance followed by the Student-Newman-Keuls method was applied to analyze differences among groups. P < 0.05 was considered statistically significant.

Results

UC-MSCs identification UC-MSCs were subcultured from passage 3 according to the method described above and were in good growth condition for flow cytometry analysis. After incubation with CD73, CD105, CD90, HLA-DR, CD34, or CD45 antibodies, flow cytometry showed the following expression patterns on the surface of isolated and cultured human UC-MSCs: CD73, CD105, and CD90 were expressed at 99.94%, 98.62%, and 98.86%, respectively, whereas HLA-DR, CD34, and CD45 showed expression rates of 0.13%, 0.36%, and 0.18%, respectively. These results indicated that the stem cells we cultured met the identification criteria for UC-MSCs (Dominici et al., 2006) (Figure 1A).

UC-MSCs attenuate the morphological and behavioral abnormalities of the rat model of MIA-associated NBI MIA-related NBI affects the ontogeny and brain morphology of neonatal rats. All 13 rats in the control group survived, whereas one of the 12 rats in the UC-MSCs relieve cerebral tissue pathological changes in the rat model of MIA-associated NBI We studied the recovery of brain injury sites after intranasal administration of UC-MSCs (Figure 3). Nissl staining showed that in the LPS + UC-MSC group, the cortical and hippocampal neuronal damage was alleviated. Neurons in the control group were sparse and were significantly stained, with neuronal cell shrinkage, chromatolysis, partial nuclear pyknosis, nuclear fragmentation, nucleolar blurring and even disappearance, and capillary zone expansion (Figure 2B). These findings suggest that injection of UC-MSCs into the mothers causes MIA-related brain damage in the offspring.
Effect of UC-MSCs on the pathological changes in the brain tissue of LPS + PBS pups. The pathological changes in the brain tissue of neonatal rats with maternal immune activation-associated neonatal brain injury (hematoxylin and eosin staining). (A) Pathological changes in neonatal rats’ hippocampus (original magnification 40×, scale bars: 250 μm). The cortical cells in the LPS group had obvious nucleoli, the number of cells was significantly reduced, and the arrangement was loose, showing a “vacuum” appearance. The hippocampal structure in the control group was more intact and cells were lined up more neatly. (B) Pathological changes in the cortex and hippocampal CA1, CA3, and DG (original magnification 200×, scale bars: 25 μm). Compared with the control group, the number of neurons in the brain cortex and hippocampus of young rats in the LPS group was significantly reduced. The neuronal nuclei were deeply stained and the nucleoli disappeared. Moreover, the neuronal structure was loose and lacked hierarchy. DG: Dentate gyrus; LPS: lipopolysaccharides; PBS: phosphate-buffered saline; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

The escape latency of the control group during the 3-day training phase, especially during the first day, showed a more regular linear arrangement along the specific entorhinal distribution pattern (A). In the Morris water maze test, we found that compared with the control group, pups in the LPS + UC-MSC group showed hindlimb drops, whereas pups in the LPS + PBS group showed hindlimb drops (Figure 4G; p = 0.006). However, in the more difficult round balance beam test, there were no significant differences in hindlimb drops between the control and LPS + PBS groups (p = 0.095) and between the LPS + PBS and LPS + UC-MSC groups (p = 0.799). These results suggest that UC-MSC treatment improved the cognitive, learning, and memory impairments caused by LPS injection during pregnancy in young rats, especially fast learning and memory. However, the effects of this treatment on motor exploration and balance of neonatal rats are still unclear and need further research.

**Astrocyte activation is related to MIA-associated NBI, and UC-MSC treatment suppresses DAAs in neonatal rats**

**Expression levels and distribution patterns of GFAP**

We performed immunofluorescence to determine the expression levels and distribution patterns of MIA-related GFAP-positive astrocytes in the brain. Immunofluorescence staining showed that many activated astrocytes infiltrated and synaptic extensions were clearly seen in the cortex of newborn rats affected by LPS during pregnancy, with a similar pattern in the CA1, CA3, and DG areas of the hippocampus. However, the fluorescence intensity of GFAP-positive activated astrocytes in the cortex and hippocampus was decreased after UC-MSC treatment (Figure 5A–D). In the cortex, GFAP-positive activated astrocytes were arranged in a centripetal layer after MIA injury. In the hippocampus, GFAP-positive astrocytes were dispersed around hippocampal neurons in the damaged area, with a relatively uniform distribution pattern (Figure 5F). In the UC-MSC group, GFAP-positive astrocytes showed a more regular linear arrangement along the specific entorhinal cortex (EC) projection pathway, with the anatomical structure suspected to align with the perforant path/CA1 indirect pathway (Figure 5G).
Effects of UC-MSCs on the immunopositivity of inflammatory cytokines

In the LPS + PBS group, IL-6 (green, stained with FITC) was highly expressed in the cortex of the LPS + PBS group. The immunopositivity of IL-1β in the cortex was similar to that in the LPS + PBS, whereas in the hippocampus it was lower. Original magnification 100×, scale bars: 75 μm. DAPI: 4′,6-Diamidino-2-phenylindole; DG: dentate gyrus; IL-1β: interleukin-1β; IL-6: interleukin-6; IP: lipopolysaccharides; PBS: phosphate-buffered saline; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

Immunopositivity of inflammatory factors in different functional brain regions

As shown in Figure 6, immunofluorescence was used to determine the immunopositivity of the inflammatory factors, IL-6 and IL-1β. In the LPS + PBS group, IL-6 and IL-1β were both highly expressed in the cortex, and IL-1β immunopositivity was slightly enhanced in the hippocampus. However, UC-MSC treatment reduced IL-6 expression in the cortex and IL-1β expression in the cortex and hippocampus.

Low expression of PTBP-1 is related to neuron regeneration and repair

Using an immunohistochemical method we examined the expression levels and distribution patterns of PTBP-1 in various parts of the brain in each group (Figure 7A and B). Compared with the control group, PTBP-1 was highly expressed in the LPS + PBS group (cortex: \( P = 0.003 \), CA1: \( P = 0.007 \)). In the cortex, PTBP-1 was abundantly expressed in the cell bodies of neurons and around neurons, with PTBP-1-positive cells distributed in a centripetal layer. Compared with the LPS + PBS group, PTBP-1 expression was decreased in the LPS + UC-MSC group (\( P = 0.025 \)), with a more orderly arrangement of PTBP-1-positive neurons. In the CA1 region, PTBP-1 expression was decreased in the LPS + UC-MSC group compared with that in the LPS + PBS group (\( P = 0.003 \)), with PTBP-1-positive cells having a more orderly arrangement and mainly distributed around damaged neurons. After LPS-induced injury, PTBP-1-positive cells in CA1, CA3, and DG showed a linear distribution along the hippocampal circuit.

Next, we used immunofluorescence to examine the distribution of GFAP-positive and PTBP-1-positive cells (Figure 8). GFAP-positive cells (\( P = 0.034 \)) and GFAP/PTBP-1-positive cells (\( P = 0.011 \)) were significantly increased in the LPS + PBS group compared with the control group, but after UC-MSC treatment, the number of both cell types decreased. In the LPS + PBS group, GFAP/PTBP-1-positive cells were linearly arranged, and PTBP-1 was expressed in the nuclei of injured neurons. In the LPS + UC-MSC group, the expression level of GFAP was reduced (\( P = 0.003 \)), and the number of GFAP/PTBP-1-positive cells was reduced compared with the LPS + PBS group (\( P = 0.007 \)). Though these cells still showed a linear arrangement, the arrangement was looser.

We also used western blot analysis to determine the expression levels of GFAP, PTBP-1, and TUJ-1 in the hippocampal region of pups after inflammatory injury and UC-MSC therapy. Compared with the control group, the protein expression levels of GFAP (\( P = 0.041 \)) and PTBP-1 (\( P = 0.002 \)) were significantly increased in the cortex of the LPS + PBS group. In contrast, compared with the LPS + PBS group, the protein expression levels of GFAP (\( P = 0.040 \)) and PTBP-1 (\( P = 0.019 \)) were significantly decreased in the cortex of the LPS + UC-MSC group. Additionally, the GFAP protein expression levels showed a decreasing trend in the hippocampus of the LPS + PBS (\( P = 0.003 \)) and LPS + UC-MSC groups (\( P = 0.000 \)), compared with the control group. Furthermore, the PTBP-1 expression levels showed an increasing trend in the hippocampus of the LPS + PBS group, compared with the control (\( P = 0.031 \)), whereas in the hippocampus of the LPS + UC-MSC group they were lower than those in the LPS + PBS group (\( P = 0.045 \); Figure 9A and B).

Finally, the protein expression of TUJ-1, which is involved in neuronal regeneration, was higher in the cortex of the LPS + PBS group than that in the control group (\( P = 0.025 \); Figure 9C). Additionally, compared with the LPS + PBS group, TUJ-1 expression was significantly higher after UC-MSC treatment (\( P = 0.012 \)), suggesting that neurons treated with UC-MSCs were in a state of regeneration after DAA activation. Moreover, after UC-MSC treatment, TUJ-1 expression in the hippocampal injury area showed an increasing trend compared with the LPS + PBS (\( P = 0.573 \)) and control groups (\( P = 0.189 \)); however, the differences among the groups were not statistically significant (Figure 9C).

**Figure 5** Effects of UC-MSCs on the distribution of GFAP-positive DAA in the cerebral cortex (A) and hippocampus CA1 (B), CA3 (C), and DG (D) of neonatal rats with maternal immune activation-associated neonatal brain injury (immunofluorescence).

In the LPS + PBS group, the GFAP (green, stained by FITC)-positive activated astrocytes (red arrows) located in the cortex were arranged in a centripetal pattern and were more evenly distributed in the hippocampus CA1, CA3, and DG. In the LPS + UC-MSC group, GFAP-positive activated astrocytes were densely distributed along the hippocampal projection pathway (green arrow). Original magnification 100×, scale bars: 100 μm. DAPI: 4′,6-Diamidino-2-phenylindole; DG: dentate gyrus; GFAP: glial fibrillary acidic protein; LPS: lipopolysaccharides; PBS: phosphate-buffered saline; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

**Figure 6** Effects of UC-MSCs on the immunopositivity of inflammatory cytokines IL-6 (A) and IL-1β (B) in the cerebral cortex and the hippocampus of neonatal rats with maternal immune activation-associated neonatal brain injury (immunofluorescence). Additionally, compared with the LPS + PBS (\( P = 0.000 \)) and LPS + UC-MSC groups (\( P = 0.007 \)), IL-1β expression in the cortex was lower than that in the LPS + PBS group. There was no significant difference between these groups in the immunopositivity of IL-6 in the hippocampus. Compared with the control group, IL-1β expression (red, stained with Alexa fluor 647) was higher in the cortex and hippocampus of the LPS + PBS group. In the LPS + UC-MSC group, the immunopositivity of IL-6 in the cortex was lower than that in the LPS + PBS group. Moreover, IL-1β expression in the hippocampus was similar to that in the LPS + PBS, whereas in the hippocampus it was lower. Original magnification 100×, scale bars: 75 μm. DAPI: 4′,6-Diamidino-2-phenylindole; DG: dentate gyrus; IL-1β: interleukin-1β; IL-6: interleukin-6; IP: lipopolysaccharides; PBS: phosphate-buffered saline; UC-MSCs: umbilical cord-derived mesenchymal stem cells.
Effect of UC-MSCs on the distribution of GFAP and PTBP-1-positive cells in the hippocampus of neonatal rats with maternal immune activation-associated neonatal brain injury.

(A) Immunofluorescence images of the distribution of PTBP-1 (red, stained with Alexa Fluor 647) and GFAP (green, stained with FITC) positive cells in the hippocampus. The number of GFAP-positive/PTBP-1-positive cells in the LPS + PBS group was significantly higher than in the control group, showing a linear arrangement. In the LPS + UC-MSC group, the number of GFAP-positive/PTBP-1-positive cells was lower than that in the LPS + PBS group. Linear arrangement was still observed, but the arrangement was looser. After LPS injury, PTBP-1 was expressed in the nucleus of the injured neurons, and GFAP surrounded the PTBP-1-positive cells. Scale bars: 100 μm (left), 20 μm (right).

(B) Quantitative results of the number of GFAP-positive cells and PTBP-1/GFAP-positive cells in the hippocampus. Data are expressed as mean ± SEM (n = 3). *p < 0.05, **p < 0.01 (one-way analysis of variance followed by Student-Newman-Keuls method).

Western blot analysis (left) was quantified (right). Data are expressed as mean ± SEM (n = 3). *p < 0.05, **p < 0.01 (one-way analysis of variance followed by the Student-Newman-Keuls method).

Discussion

Prenatal MIA caused by infection during pregnancy can cause acute and chronic changes in the structure and function of the fetal central nervous system (Estes and McAllister, 2016). Significant associations have been identified between NBI caused by infection during pregnancy and increased risks of schizophrenia (Brown, 2011), bipolar disorder (Canetta et al., 2014), and cerebral palsy (Harvey and Boksa, 2012). MIA-related brain injury is closely related to excessive activation of astrocytes and oligodendrocytes (Burda et al., 2016; Imai et al., 2018). The occurrence and progression of MIA-induced NBI may be associated with the state and extent of astrocyte activation in specific areas (e.g., cortical and hippocampal projection circuits) at different time periods (Purves-Tyson et al., 2021).

In this study, using immunofluorescence we demonstrated that astrocytes with high GFAP expression levels were located in LPS-induced damaged cortex and hippocampal projection areas and were similar in morphology to DAAs, which validated this phenomenon in animal models of brain injury development (Purves-Tyson et al., 2021).

Figure 7 | Effect of UC-MSCs on the immunopositivity and distribution of PTBP-1 in various areas of the brain of neonatal rats with maternal immune activation-associated neonatal brain injury.

(A) The distribution of PTBP-1 (green arrows) in the cerebral cortex and hippocampal CA1, CA3, and DG (scale bars: 100 μm; 50 μm in enlarged parts). Cortex: under low magnification, the expression level of PTBP-1 protein in the LPS + PBS group was higher than that in the control group. After UC-MSCs administration, the expression level of PTBP-1 protein, compared with the LPS + PBS, was lower. Under high magnification (green framed field), PTBP-1 was abundantly expressed in neurons and cell bodies around neurons, and PTBP-1-positive cells were distributed centripetally along the cortex. Hippocampus: under low magnification, the expression of PTBP-1 in the hippocampal CA1 area of the LPS + PBS group was higher than that in the control group and LPS + UC-MSC group. There was no significant difference between the control group, LPS + PBS group, and LPS + UC-MSC group in hippocampal CA3 and DG areas. Under high magnification, the PTBP-1-negative cells in the DG zone were loosely arranged and swollen in the LPS + PBS group; compared with the LPS + PBS group, the PTBP-1-positive cells and PTBP-1-negative cells in the UC-MSC group were arranged more neatly and mainly distributed around the damaged neurons (green arrow). (B) Quantitative results of PTBP-1 immunopositivity. Data are expressed as mean ± SEM (n = 3). *p < 0.05, **p < 0.01 (one-way analysis of variance followed by Student-Newman-Keuls method). DAPI: 4′, 6-Diamidino-2-phenylindole; DG: dentate gyrus; GFAP: glial fibrillary acidic protein; LPS: lipopolysaccharides; PBS: phosphate-buffered saline; PTBP-1: polypyrimidine tract-binding protein-1; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

Figure 8 | Effect of UC-MSCs on the distribution of GFAP and PTBP-1-positive cells in the hippocampus of neonatal rats with maternal immune activation-associated neonatal brain injury.

(A) The distribution of GFAP (green) and PTBP-1 (red) in the hippocampus of neonatal rats with maternal immune activation-associated neonatal brain injury. Western blot analysis (left) was quantified (right). Data are expressed as mean ± SEM (n = 3). *p < 0.05, **p < 0.01 (one-way analysis of variance followed by Student-Newman-Keuls method). DAPI: 4′, 6-Diamidino-2-phenylindole; DG: dentate gyrus; GFAP: glial fibrillary acidic protein; LPS: lipopolysaccharides; PBS: phosphate-buffered saline; PTBP-1: polypyrimidine tract-binding protein-1; TUJ-1: neuron-specific class III β-tubulin; UC-MSCs: umbilical cord-derived mesenchymal stem cells.
There were several limitations to our study that should be noted. Our research also supports previous evidence related to the therapeutic effectiveness of stem cells, especially their anti-inflammatory effects and the feasibility of their intranasal delivery into the brain (Jiao et al., 2019, 2021). The TUJ-1 expression pattern suggests that this relative decrease in PTBP expression may be involved in the process of dynamic transdifferentiation of astrocytes or in the plasticity repair of neurons; however, these specific processes require further study.

We also investigated morphological and behavioral abnormalities in pups with MIA. The present study could be a direction for future research. In summary, PTBP-1 may be a key protein in the regulation of brain inflammatory damage by DAAs, as well as neuronal regeneration and neuroplasticity repair after MIA during pregnancy under both physiological and pathological conditions. The correlation cannot be a direct linear effect relationship. In the latest neurodegenerative disease model studies, PTBP-1 protein expression sites have been shown to be distributed around dopaminergic neurons in the midbrain, with expression levels closely related to the number and function of GFAP-positive astrocytes by inhibiting PTBP-1 expression levels were either decreased, or their high expression sites were reduced, suggesting that PTBP-1 may be involved in the regulation of brain inflammatory response and in GFAP-positive astrocyte activation. We speculate that UC-MSCs may reduce inflammation in the brain, and the connections and mechanisms by which astrocytes and microglia respond after injury remain to be further elucidated.

Conflict of interest: We have declared no conflict of interest.

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Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Additional file: Additional file 1: Informed consent form (Chinese).
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