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Full-length Article

Xenograft of human umbilical mesenchymal stem cells from Wharton's jelly as a potential therapy for rat pilocarpine-induced epilepsy

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ABSTRACT

We evaluated the effects of intra-hippocampal transplantation of human umbilical mesenchymal stem cells (HUMSCs) on pilocarpine-treated rats. Sprague–Dawley rats were divided into the following three groups: (1) a normal group of rats receiving only PBS, (2) a status epilepticus (SE) group of rats with pilocarpine-induced SE and PBS injected into the hippocampi, and (3) a SE + HUMSC group of SE rats with HUMSC transplantation. Spontaneous recurrent motor seizures (SRMS) were monitored using simultaneous video and electroencephalographic recordings at two to four weeks after SE induction. The results showed that the number of SRMS within two to four weeks after SE was significantly decreased in SE + HUMSCs rats compared with SE rats. All of the rats were sacrificed on Day 29 after SE. Hippocampal morphology and volume were evaluated using Nissl staining and magnetic resonance imaging. The results showed that the volume of the dorsal hippocampus was smaller in SE rats compared with normal and SE + HUMSCs rats. The pyramidal neuron loss in CA1 and CA3 regions was more severe in the SE rats than in normal and SE + HUMSCs rats. No significant differences were found in the hippocampal neuronal loss or in the number of dentate GABAergic neurons between normal and SE + HUMSCs rats. Compared with the SE rats, the SE + HUMSCs rats exhibited a suppression of astrocyte activity and aberrant mossy fiber sprouting. Implanted HUMSCs survived in the hippocampus and released cytokines, including FGF-6, amphiregulin, glucocorticoid-induced tumor necrosis factors receptor (GITR), MIP-3β, and osteoprotegerin. In an *in vitro* study, exposure of cortical neurons to glutamate showed a significant decrease in cell viability, which was preventable by co-culturing with HUMSCs. Above all, the expression of human osteoprotegerin and amphiregulin were significantly increased in the media of the co-culture of neurons and HUMSCs. Our results demonstrate the therapeutic benefits of HUMSC transplantation for the development of epilepsy, which are likely due to the ability of the cells to produce neuroprotective and anti-inflammatory cytokines. Thus, HUMSC transplantation may be an effective therapy in the future.

1. Introduction

Temporal lobe epilepsy (TLE) is a chronic disease characterized by spontaneous, progressive seizures. In many patients, TLE is initiated by brain injury, strokes, tumors or status epilepticus (SE), which is often followed by a latency period of 5–10 years before

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the onset of spontaneous recurrent motor seizure (SRMS) (Chang and Lowenstein, 2003; O'Dell et al., 2012; Sharma et al., 2007). SE is defined as a continuous seizure activity lasting more than 5 min (Brophy et al., 2012). SRMS is the repeated unprovoked seizures that occurs in the chronic phase of epilepsy (Hattiangady and Shetty, 2008). The main physiopathological findings observed in TLE are neuronal loss, reactive gliosis, aberrant mossy fiber sprouting and spontaneous recurrent motor seizure (SRMS) (Acharya et al., 2008; Carpentino et al., 2008; Rao et al., 2006). Antiepileptic drugs fail to well-control seizures in approximately 30% of epilepsy patients. Stem cell grafting is considered to be a promising alternative approach for a better treatment of epilepsy (French et al., 2004; Nilsen and Cock, 2004).

There are two possible mechanisms of stem cell transplantation in the treatment of epilepsy. In one mechanism, the transplanted stem cells may replace the cells lost by trans-differentiation. Stem cells possess self-renewal properties, and the ability to differentiate into different cell types, which may replace lost cells or specific neurons during epilepsy, such as inhibitory interneurons (Roper and Steindler, 2013). On the other hand, neuroprotective or anti-inflammatory cytokines are released from stem cells, which are involved in the repair of autoimmune encephalomyelitis, amyotrophic lateral sclerosis, stroke and spinal cord injury (Einstein et al., 2007; Lin et al., 2011; Vercelli et al., 2008; Yang et al., 2008). To date, using stem cells to replace the cells that are lost during the course of epileptogenesis has been considered an effective strategy in the treatment of epilepsy (Cunningham et al., 2014; Hunt et al., 2013). However, little is known on the exact trophic mechanisms of stem cell therapy in epilepsy.

Human mesenchymal cells from Wharton's jelly of the umbilical cord are obtained from medical waste after delivery and therefore carry little ethical concerns. These human umbilical mesenchymal stem cells (HUMSCs) can differentiate into neurogenic, osteogenic, chondrogenic, adipogenic, and myogenic cells *in vitro* (Fu et al., 2004; Mitchell et al., 2003; Wang et al., 2004). Our previous studies have shown that the engrafted HUMSCs are viable in ischemia cortex, striatum, and spinal cord of rats without the need for immunological suppression (Fu et al., 2006; Lin et al., 2011; Yang et al., 2008). HUMSC transplantation effectively protects neurons from atrophy, prevents inflammation, and ameliorates motor performance deterioration, which is likely due to the ability of the cells to produce growth-promoting factors (Lin et al., 2011; Yang et al., 2008). In addition to their effects in the central nervous system, HUMSC transplants in rats with liver fibrosis and peritoneal fibrosis also exhibit promising therapeutic potentials by means of cytokine release (Tsai et al., 2009). These results indicate that HUMSCs possess the ability of long-term survival, and maintain their functions within various host organs of the rat, suggesting that HUMSCs are a good stem cell source for xenotransplantation.

To evaluate whether HUMSC transplantation at the acute phase of brain damage prevents or alleviates chronic epilepsy, HUMSCs were isolated from Wharton's jelly of human umbilical cords and transplanted into hippocampi of pilocarpine-treated rats. We then investigated the effects HUMSCs of on seizure frequency and duration and cognitive outcome in chronic epilepsy. The results showed that the transplanted cells remained viable and functional for one month, effectively ameliorating the hippocampal injury, decreasing glial activation, and suppressing circuitry reorganization.

2. Materials and methods

The use of human umbilical cord and laboratory animals in this study was approved by the Research Ethics Committee at Taipei Veterans General Hospital and the Animal Research Committee of the College of Medicine at National Yang-Ming University.

2.1. Animals

Six-week-old male Sprague–Dawley rats (230–270 g) were used in this study. All of the rats were sacrificed four weeks after surgery. All of the animals were kept in individual cages under environmentally controlled conditions (12-h light/dark cycle) and were given food and drinking water *ad libitum*. The animals were divided into the three following groups: (1) the normal group, where rats received only an intraperitoneal injection of the phosphate buffer saline vehicle (PBS; pH 7.4) in place of pilocarpine ($n = 16$); (2) the status epilepticus group (referred to as the *SE* group), where SE in rats was induced by an intraperitoneal injection of pilocarpine in PBS ($n = 28$); and (3) the SE + HUMSCs group, where rats received both pilocarpine and transplanted HUMSCs ($n = 32$).

2.2. Isolation and preparation of HUMSCs

Human umbilical cords were collected in Hanks' balanced salt solution (HBSS) (14185-052; Gibco) at 4 °C. Following disinfection with 75% ethanol for 30 s, the umbilical cord vessels were cleared off in HBSS. The mesenchymal tissues in Wharton's jelly were then diced into cubes of 0.5 cm in length on each side, and centrifuged at 250×*g* for 5 min. After removal of the supernatant fraction, the precipitate containing the mesenchymal tissue was washed with serum-free Dulbecco's modified Eagle's medium (DMEM) (12100-046; Gibco) and centrifuged again at 250×*g* for another 5 min. After aspirating off the supernatant fraction, the mesenchymal tissue in the precipitate was treated with collagenase (17100-017; Gibco) at 37 °C for 18 h, washed, and further digested with 2.5% trypsin (15090-046; Gibco) at 37 °C for 30 min. Fetal bovine serum (FBS) (26140079; Gibco) was added to neutralize the excess trypsin. The dissociated mesenchymal cells were cultured in 10% FBS-DMEM.

2.3. Primary culture of rat cortical neurons

The postnatal one-day-old rats were placed under anesthesia, and cerebral cortices were excised in a sterile laminar flow chamber. The cortices were then collected in a centrifuge tube containing Ca^{2+}/Mg^{2+}-free Hank's buffer, and centrifuged at 900 rpm for 5 min. The supernatant was discarded. DMEM containing 10% FBS was then added to the pellet. The suspension was completely mixed by triturating 15 times with a sterile glass pipette. The cortical neurons were seeded at a density of 5×10^3 cells/cm^2. To inhibit the growth of glial cells, 20-μM cytosineβ-D-arabino-furanoside (C-6645; Sigma) was added to each well after 24 h. The fetal serum-containing culture medium was replaced with serum-free DMEM on the fifth day of culture. The culture systems of cortical neurons alone or neurons and HUMSCs co-cultures were performed on Day 5 as well.

2.4. Culture systems of rat cortical neurons alone or neurons and HUMSCs co-cultures

To explore the effect of HUMSCs on cortical neuron damage that is induced by glutamate, cortical neurons were cultured alone or with HUMSCs in a special transwell system. The co-culture system consisted of upper and lower chambers separated by a distance not physically traversable by the cells. The chambers, however, shared the same medium, which covered both cultures, thus allowing access to both cultures by humoral factors. Forming the bottom of the upper chamber was a porous membrane with multiple pores

with an 8-μm diameter, which allowed medium to cross the membrane without the actual mixing of the cells.

Primary HUMSCs were cultured in the upper chamber of the transwell co-culture system, with cortical neurons cultured in the lower chamber. The cortical neurons that were cultured alone or cultured with HUMSCs were cultured in serum-free medium for two days and then treated with vehicle or 1-mM glutamate for 1 h. The medium was replaced with new serum-free medium to remove glutamate, and these cortical neuron and HUMSC cultures continued for 48 h.

2.5. The assessment of neuron viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay measures the mitochondrial activity of viable cells by quantifying the conversion of the tetrazolium salt to its formazan product (Aras et al., 2008). Cortical neurons were treated with MTT solution (final concentration 1 mg/ml; M5655; Sigma) to determine the proportion of viable cells.

NeuroTrace is a fluorescent Nissl stain that only labels neurons (Quinn et al., 1995). Cortical neurons were treated with NeuroTrace solution (N-21480; Molecular Probe) to visualize and estimate neuron viability (Lazo et al., 2010).

2.6. Quantification of cytokine levels in vitro

Protein samples from the media of neurons alone or neuron and HUMSC co-cultures lysed in a protein lysis buffer containing phosphatase and protease inhibitors were collected for an enzyme-linked immunosorbent assay (ELISA). Human amphiregulin and osteoprotegerin in the media were determined using the Quantikine ELISA Kit (ELH-OPG and ELH-AR; RayBiotech) according to the manufacturer's protocol.

2.7. Pilocarpine-induced SE

Rats were pretreated with anti-muscarinic drug scopolamine (1 mg/kg, intraperitoneal; S2250; Sigma) to minimize peripheral muscarinic effects. Thirty minutes after scopolamine, rats were injected with pilocarpine (330 mg/kg, intraperitoneally; P6503; Sigma) to induce SE. Rats developed SE within 5–30 min. To limit the duration of SE and the extent of the damage in the hippocampus, diazepam (10–20 mg/kg; CCPC) was injected intraperitoneally 90 min after the onset of SE (Biagini et al., 2006). The severity of convulsive seizures were classified according to Racine's Scale (Racine, 1972) as follows: Stage 1, mouth and focal movements; Stage 2, head nodding; Stage 3, contra-lateral forelimb clonus; Stage 4, symmetrical forelimb clonus with rearing; and Stage 5, severe seizure with rearing and falling. All of the surgeries were performed with the animals under anesthesia with isoflurane (Baxter Healthcare). The rat number totaled 155. Eighty-three out of 155 rats died during SE. Twelve rats displayed Stage 0–1 of the Racine's Scale. Sixty rats displaying a minimum of Stage 3 during this observation period were selected for further experiments in either the SE or SE + HUMSCs group.

2.8. Transplantation of HUMSCs

One day after SE induction, rats were randomly selected for PBS injection or cell transplantation. A total of 10^5 HUMSCs were stereotaxically injected into the bilateral dorsal hippocampi (AP: −4.8 mm; ML: ±4.8 mm; DV: −7.0 mm; total cell number: 2×10^5 cells) via a 10-μl Hamilton syringe with a 26-gauge needle. The syringe was left in place for a few minutes to minimize backing up of cells up the needle track (Gage et al., 1995). No immunosuppressive medications were administered to any animal in this study. In the SE groups, 5 μl of PBS was injected instead of HUMSCs.

2.9. Electroencephalography (EEG) monitoring and analysis

EEG/video recordings were employed to monitor spontaneous motor seizures. At one day after SE induction ($n = 6$ in SE or SE + HUMSCs groups), epidural recording electrodes were implanted into the right and left lateral parietal association cortices (AP: −4.0 mm, ML: ±3.0 mm) (Wang et al., 2009). After allowing one week for recovery from surgery, the rats underwent EEG/video monitoring from two to four weeks post SE. The recording duration for each rat was 9 h/week (totaling 27 h over three weeks). All of the EEG analog data were collected using a multi-channel neurophysiology system (Plexon Inc.). Simultaneous video recording was carried out with a camera recorder (Logitech). EEG signals were redigitalized at 250 Hz and analyzed according to autocorrelation methods based on those of White et al. (2006). EEG-detected spontaneous seizures were further confirmed using simultaneous video imaging.

2.10. Magnetic resonance imaging (MRI)

T2-weighted images of the hippocampus regions in the rats ($n = 4$/group) were taken using a high-resolution 7T MRI system (Bruker Biospin). MRI was performed on each rat at one week before SE and one, eight, 15, 22, and 29 days afterwards. Settings of the images were a field-of-view (FOV) (cm) = 2.5 × 2.5, thickness (mm) = 0.5 or 1, a slice sep. (mm) = 0.5 or 1, and a Matrix size = 256 × 256 resolution (um). The imaging parameters used were a number of excitation (NEX) = 12, a TR (ms) = 2522.824 or 3000, and a TE (ms) = 33. Images of serial coronal sections from dorsal to ventral hippocampi were obtained at 0.5 mm intervals and analyzed using the software *Image J*. In the MRIs, white areas in the hippocampus and lateral ventricles indicated edema.

2.11. Histological and immunohistochemical examinations

At four weeks after the onset of SE, the rats were sacrificed and transcardially perfused with 4% paraformaldehyde ($n = 6$, 8, and 9 in normal, SE, and SE + HUMSCs groups, respectively). The brains were post-fixed in the same fixation solution for 24 h and then in 30% sucrose solution in PBS overnight for cryoprotection. Cryostat sections were cut at a 30-μm thicknesses with every 10th of the hippocampal sections selected for histological and immunohistochemical analyses following staining with Nissl or the appropriate antibodies. For histological examination, hippocampal sections were incubated in PBS containing 0.5% H_2O_2 for 20 min to block endogenous peroxidases. After rinsing in PBS, the specimens were incubated in blocking solution with 5% normal goat serum and 3% bovine serum albumin in 0.1 M PBS for 30 min to block any non-specific binding. Following the removal of the blocking solution, primary antibodies, including mouse anti-NeuN (MAB377; Millipore Corporation), mouse anti-Parvalbumin (MAB1572; Millipore Corporation), rabbit anti-GFAP (AB5804, Millipore Corporation) and mouse anti-ED1 (MAB1435; Millipore Corporation), were added by dilution in blocking solution to the appropriate concentration and the specimens were incubated overnight at 4 °C. Following three washes in PBS, appropriate biotinylated secondary antibodies were added and allowed to stain for 1 h. Following three more washes with PBS, the specimens were reacted with an ABC Complex Kit (PK-4000, Vector Laboratories) at room temperature for 1 h. Immunolabeled sections were developed with 3,3′-diaminobenzidine tetrahydrochloride hydrate (D5637; Sigma) as the chromogen with 0.01% H_2O_2 in 50-mM Tris buffer. Specimens

were finally dehydrated with alcohol and coverslipped with Permount (SP15-500; Fisher Scientific).

2.12. Nissl staining

Cryostat sections were mounted on gelatin-coated slides. Following immersion in 1% Nissl (C5042; Sigma), the sections were dehydrated in ethanol, cleared in xylene (247642; Sigma), and coverslipped with Permount.

2.13. Timm's staining

Timm's staining is based on the staining of Zn^{2+}-containing mossy fibers by a sulfide/silver stain. The severity of the MFS were evaluated based on mossy fiber sprouting in the hippocampus. Under deep anesthesia, rats ($n = 4$/group) were perfused with 200 ml of 12% sodium sulfide hydrate (13,468; Sigma) in 0.1 M sodium phosphate solution (S9638; Sigma), and followed by 200 ml of 2% paraformaldehyde (16,005; Sigma) and 1.25% glutardialdehyde (GL01701000; Scharlau) in 0.1-M phosphate buffer (PB). Perfused brains were fixed in the same fixation solution for 24 h and then placed in 30% sucrose in 0.1-M PB until the brains sank to the bottom of the tubes. Serial coronal sections through the entire hippocampus were cut at a thickness of 30-μm with the aid of a cryostat and processed for Timm's staining. The sections were stained in a solution containing 30% gum Arabic (G9752; Sigma), 1.7% hydroquinone (H9003; Sigma), 2.3% sodium citrate (S4641; Sigma), 2.5% citrate acid (C0759; Sigma) and 1.05 mg/ml silver nitrate (S6506; Sigma) for 1 h (Hsu and Buzsaki, 1993). After washing with H_2O, sections were mounted with Permount.

2.14. Tracing of HUMSCs using bisbenzimide treatment and anti-human nuclear Ag immunohistochemistry

To trace the viability and distribution of the implanted HUMSCs, the cells were labeled with 1 g/ml of bisbenzimide (B2883; Sigma) for 48 h before trypsinization and transplantation. Following sacrifice, rats ($n = 3$) were perfused with 4% paraformaldehyde (Un2213; Merck) and 7.5% picric acid (925-40; Sigma) in 0.1-M phosphate buffer (PB). The brain tissues were post-fixed in the same fixative for 24 h and cryo-protected in 30% sucrose in 0.1-M PB. Following sedimentation, the tissue was embedded in optimum cutting temperature (OCT) compound, frozen, and cryosectioned at 30-μm in a cryostat (−20 °C). Frozen sections were thaw-mounted onto coated glass slides, and directly observed and photographed under fluorescence microscope. In addition, immunostaining using anti-human specific nuclear antigen was performed to trace the survival of HUMSCs. Tissue sections were first reacted with a primary antibody (MAB1281; Millipore Corporation) and at 4 °C for 18 h, washed with 0.1-M PBS, and reacted with secondary antibodies (biotin-conjugated goat anti-mouse-IgG; Sigma) at room temperature for 1 h. After the chromogenic reaction, the sections were coverslipped and observed under a microscope.

2.15. Human cytokine array

To elucidate which human cytokines were involved in the suppression of the SRMS, a human protein cytokine kit (AAH-CYT-2000, RayBio® Human Cytokine Antibody Array C Series 2000, RayBiotech) was used to screen the expression of 174 human cytokines ($n = 3$/group). Rats were deeply anesthetized and decapitated at 28 days after HUMSC transplantation. Hippocampi were homogenized in lysis buffer and centrifuged at 1500×g to separate cell debris. The supernatant was harvested and then incubated for 2 h at room temperature with membranes containing an array of human cytokine antibodies. The levels of cytokine expression were determined by the intensities of immunoreactivity as relative to that of the standard controls using enhanced chemiluminescence according to the manufacturer's instructions.

2.16. Reverse transcription PCR for detecting human NeuN and GFAP in the hippocampus

Total RNA was freshly isolated from the rat hippocampi using the TRIzol Reagent (Invitrogen) ($n = 3$/group), and reverse transcribed into complementary DNA with SuperScript III (18,080, Invitrogen). Specific primers for PCR were designed to detect only human NeuN and GFAP with primer express software (Vector NTI Software, Invitrogen). PCR products were amplified with 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s), and resolved on a 2% agarose gel. Human hippocampus was used as a positive control. The primer sequences were as follows:

Human NeuN
Sense: 5′-ATCCAGTGGTCGGCGCAGTCTAC-3′
Antisense: 5′-TACGGGTCGGCAGCTGCGTA-3′
Human GFAP
Sense: 5′-TCCACTTCCTCCTCCTCCACGA-3′
Antisense: 5′-AACTTGCACACGGCGCAGGT-3′
Human GAPDH
Sense: 5′-TTCCACCCATGGCAAATTCCATGG-3′
Antisense: 5′-GGTCAGGTCCACCACTGACACG-3′
Rat GAPDH
Sense: 5′-CTCTACCCACGGCAAGTTCAAC-3′
Antisense: 5′-GGTGAAGACGCCAGTAGACTCCA-3′
Human and rat β-actin (Jones et al., 1999; Kowalewski et al., 2010)
Sense: 5′-TTGTAACCAACTGGGACGATATGG-3′
Antisense: 5′-GATCTTGATCTTCATGGTGCTAGG-3′

2.17. Quantification and statistical analyses

The statistics software SPSS 17.0 was used for statistical analyses. Data were expressed as the mean ± SEM for each group. Data were analyzed by an ANOVA followed by post hoc Bonferroni analyses. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. HUMSC transplantation attenuated the incidence and duration of spontaneous recurrent seizures in the chronic phase

To examine the effects of HUMSC transplantation on pilocarpine-induced SE, seizures were monitored both by video and EEG recordings from two to four week after PBS injection or HUMSC implantation. SE induced in the pilocarpine treated rats was characterized by continuous motor-limbic seizures lasting in excess of 90 min. Surface EEG that was recorded from the brain cortex showed seizure spikes during SRMS in both the SE and SE + HUMSCs groups (Fig. 1A and B), with convulsive seizure detection based on the effective algorithms designed by White AM (White et al., 2006). The results indicated that the percentages of occurrence were significantly different between the two groups with five out of six (83%) rats in the SE groups developing SRMS, while only three out of six (50%) rats in the SE + HUMSCs group developed SRMS. The onset of the seizure was also delayed by HUMSCs transplantation from the 2nd week after pilocarpine treatment in the SE rats to the 3rd week in the SE + HUMSCs rats (Fig. 1C). Significant differences were also present between the two groups in incidence and duration. The total number of seizures in the SE rats was significantly greater by 5.33 ± 1.52 compared

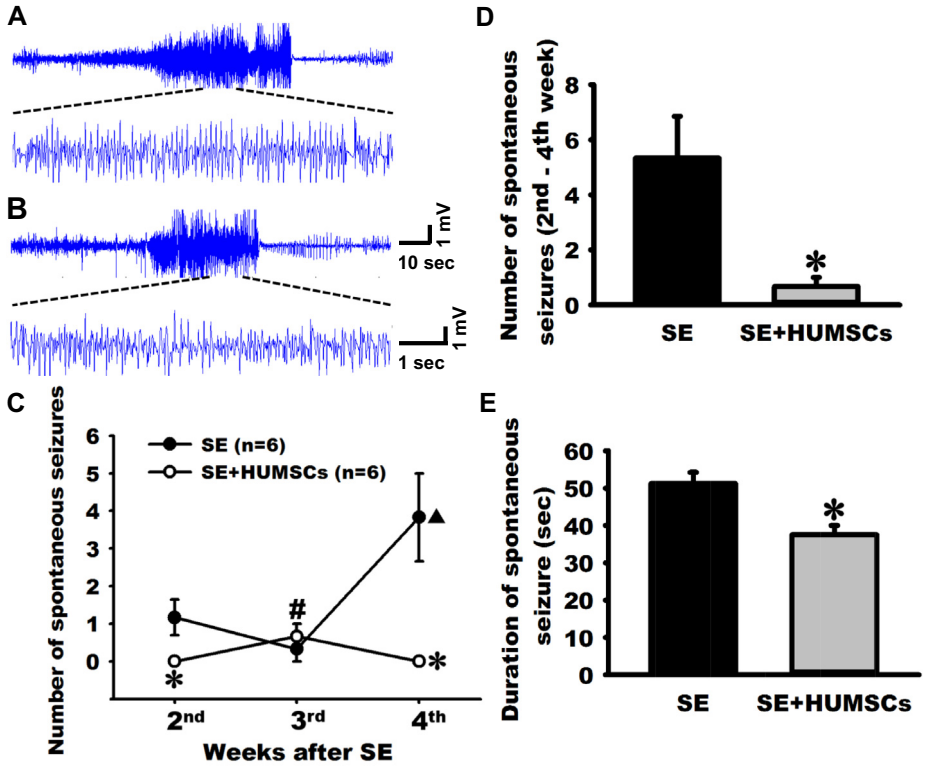

Fig. 1. Antiepileptic effects of HUMSC transplantation on pilocarpine-induced SE. Representative EEG tracings of SE (A) and SE + HUMSC (B) rats. Incidence (C), total numbers (D) and duration (E) of spontaneous seizures between the 2nd and 4th weeks in the two groups of rats. HUMSC transplantation significantly decreased all of these measures. $^{*}p < 0.05$, compared with SE rats at the same day. $^{\#}p < 0.05$, compared with the value obtained in the 2nd week in the SE group. ▲ $p < 0.05$, compared with the value observed in the 3rd week in the SE group.

with 0.67 ± 0.33 in the SE + HUMSCs rats (Fig. 1C and D). Seizure durations were also significantly longer in the SE rats than in the SE + HUMSCs rats (Fig. 1E). Taken together, HUMSCs transplantation attenuated pilocarpine-induced SE in terms of onset, incidence, and duration.

3.2. HUMSCs transplantation reduced brain edema in chronic epilepsy

Other pathological changes after pilocarpine-induced SE such as brain edema were evaluated using MRI (Fig. 2A and B). There were hardly any detectable edema areas in the rat brain before pilocarpine injection (pictures not shown). In the SE group, brain edema appeared in the hippocampus, lateral ventricles, thalamus, and piriform cortex one day after SE. Significantly increased edema was found in the hippocampus, lateral ventricles, and the 3rd ventricle eight days after SE induction. Brain edema reached a plateau at 15 days and persisted up to 29 days in the SE group (Fig. 2A). In SE + HUMSCs group, lateral ventricle edema appeared one day after SE; however, brain edema at eight days was similar to that at one day. Starting from 15 days after SE, edema in the ventricle and hippocampus were increased and persisted up to 29 days in the SE + HUMSCs group (Fig. 2B). In addition, HUMSCs transplantation significantly attenuated the edema in the ventricles, hippocampus, and piriform cortex on Days 8 and 29 post-SE (Fig. 2D). In summary, the brain edema was attenuated significantly by the transplantation of HUMSCs into the rat hippocampi.

3.3. HUMSC transplantation reduced hippocampal cytoarchitecture changes in chronic epilepsy

Possible changes in the cytoarchitecture associated with pilocarpine were examined histo-morphologically on Nissl stained coronal sections that were obtained following the sacrifice of the rats 29 days after pilocarpine-induced SE. Consistent with MRI T2 signal results, the lateral ventricles were significantly enlarged in the SE group than those in the SE + HUMSC group (Fig. 2C). Morphology of the dorsal hippocampus was abnormal, and the dorsal hippocampal volume in the SE group was significantly decreased compared with those in the other two groups (Fig. 2C and E). There was no significant difference in hippocampal volume between the normal and the SE + HUMSC groups (Fig. 2E), suggesting a protective role for the transplanted HUMSCs in preventing brain edema and hippocampal atrophy resulting from pilocarpine-induced SE.

3.4. HUMSCs transplantation preserved the integrity of the hippocampal pyramidal neurons and inhibitory circuitry in chronic epilepsy

The effects of pilocarpine treatment and HUMSCs transplantation on the neuronal circuitry in the hippocampus were evaluated immunohistochemically by staining the pyramidal and GABAergic interneurons with anti-NeuN and anti-parvalbumin. Quantitative calculation of pyramidal neurons in the dorsal hippocampus demonstrated that the number of pyramidal neurons was decreased in the CA1 and CA3 regions in the SE compared with those of the normal group (Fig. 3A, B, and G). The loss of pyramidal neurons in the CA1 and CA3 regions induced by pilocarpine injection was attenuated by HUMSC transplantation (Fig. 3C and G). There was barely a detectable change in the number of hilar cells in the dentate gyrus in all three groups (Fig. 3G).

Compared with the normal rats, pilocarpine-induced SE caused loss of GABAergic interneurons in the hippocampal CA1 and CA3 regions (Fig. 3D, E, and H). Such losses were attenuated by HUMSC transplantation (Fig. 3F and H). These results suggest that HUMSCs transplantation exhibited neuroprotective effects on the surviving pyramidal cells and inhibitory interneurons.

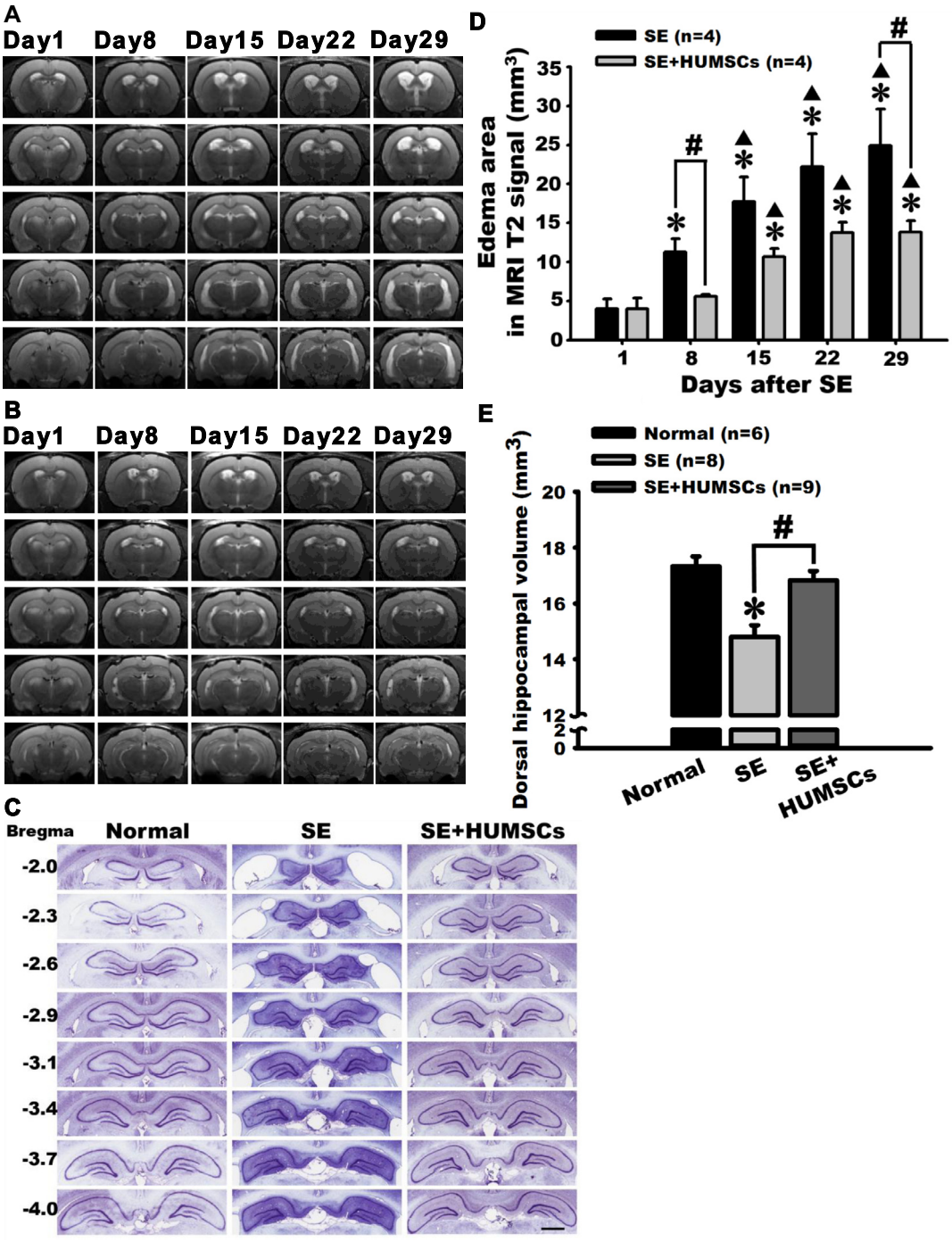

Fig. 2. Brain edema examined using MRI and Nissl staining. Magnetic resonance imaging T2-weighted images obtained from SE (A) and SE + HUMSC rats (B). (A) T2-weighted signals showing increases in the hippocampus one day after SE. Hippocampal and ventricular edema becoming apparent in SE group at eight days following a SE induction. (B) HUMSC transplantation delayed the appearance of edema following SE. (D) Quantitative data between SE and SE + HUMSC rats showing an effective reduction of brain edema in the lateral ventricles and a partial hippocampal region for the HUMSC transplanted group. * < 0.05, the edema volume compared with the value obtained at one day after SE in the same group. ▲ $p < 0.05$, the edema volume compared with the value obtained at eight days after SE in the same group. $^{\#}p < 0.05$, edema volume compared with SE group at the same day. (C) Cytoarchitecture of the hippocampus by Nissl staining in normal, SE and SE + HUMSC rats 29 days after pilocarpine-induced SE. (E) Dorsal hippocampal volume was calculated using Nissl staining. In comparison to the normal hippocampus, SE rats showed a lateral ventricle edema and dorsal hippocampal atrophy, which was prevented by HUMSC transplantation. * < 0.05, compared with the normal group. $^{\#}p < 0.05$, comparison between the SE and SE + HUMSC groups. Scale bars = 200 μm (C).

3.5. HUMSCs transplantation inhibited astrocyte and microglial cells activation in the chronic epilepsy

To further investigate the effects of transplanted HUMSCs on brain inflammation induced by pilocarpine-induced SE, the expression of astrocyte and microglial cells in the three groups were evaluated. In the normal brain, astrocytes are only minimally activated (Fig. 4A and D). The fiber density of astrocytes was significantly increased in the hippocampal CA1, CA3, and dentate gyrus regions only in the SE group (Fig. 4B and D). The levels of astrocyte

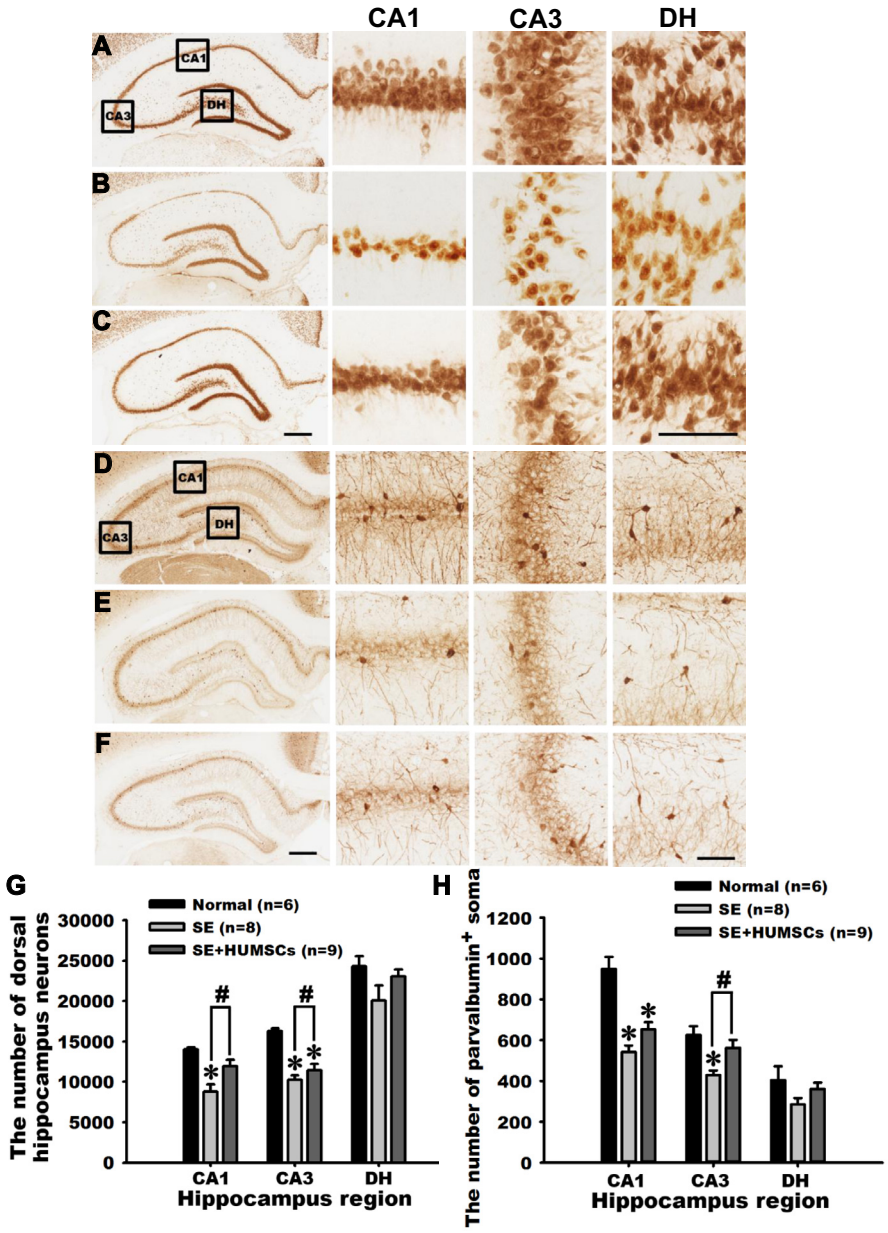

Fig. 3. HUMSCs transplantation alleviates pyramidal neurons and interneurons loss in the hippocampus after pilocarpine-induced SE. The distribution of pyramidal neurons in the hippocampal regions as visualized using anti-NeuN immunostaining in normal rats (A), SE (B) and SE + HUMSC rats (C) at 29 days after SE. (G) Bar graphs showing the number of pyramidal neurons in the hippocampal regions in the three groups. HUMSC transplantation attenuated SE-induced neuronal losses in the CA1 and CA3 regions (A–C). No significant differences between the three groups were observed in the DH region (A–C). Photomicrographs showing the distribution of GABAergic inhibitory interneurons as visualized using anti-parvalbumin in the normal (D), SE (E) and SE + HUMSC rats (F). (H) Histograms showing the number of parvalbumin positive neuronal soma in hippocampal regions in the three groups of rats. HUMSC transplantation attenuated SE-induced parvalbumin-positive cell loss in the CA1 and CA3 regions. No significant differences between the three groups were observed in the DH region. Scale bars = 500 μm (A–F); 100 μm (in CA1, CA3, and DH). * < 0.05, compared with normal rat in the same region. $^{\#}p < 0.05$, comparison between SE and SE + HUMSC groups.

activity in SE + HUMSCs rats were similar to those in the normal rats (Fig. 4A and C). Transplanted HUMSCs effectively reduced the pilocarpine-induced astrogliosis (Fig. 4D).

In the normal brain, microglial cells were scarcely detected (pictures not shown). Marked ED1 revealed significantly more activated microglial cells in the hippocampus of the SE group (Fig. 4E and F).

In the SE + HUMSCs group, there were some activated microglial cells in the hippocampus. There were fewer activated microglial cells after stem cell transplantation compared with those of the SE group (Fig. 4G and H). These results demonstrated that HUMSCs transplantation suppressed pilocarpine-induced astrogliosis and brain inflammation.

3.6. HUMSCs transplantation attenuated mossy fiber sprouting in chronic epilepsy

To investigate whether pilocarpine-induced SE changes the neuronal circuitry, Timm's staining was used to monitor mossy fibers, which are the axons of hippocampal dentate granule cells that extend their processes into the inner molecular layer (IML), the dentate granule cell layer and the CA3 pyramidal cell layer. Aberrant mossy fiber sprouting was found in the chronically injured hippocampus (Okazaki et al., 1995; Shetty and Turner, 1997; Shetty et al., 2003). Our results indicated that the mossy fiber sprouting toward the inner molecular layer significantly increased in the SE rats compared with that in the normal rats

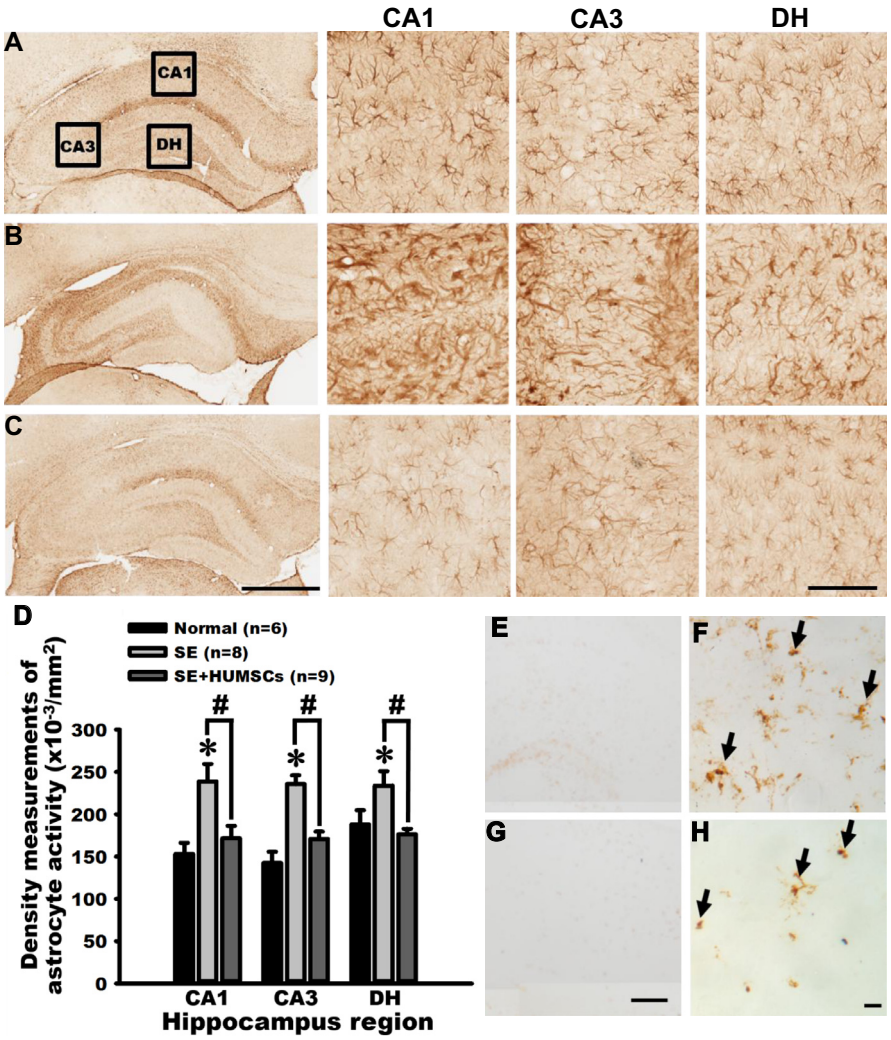

Fig. 4. HUMSC transplantation attenuates the activation of neuroglial cells in the hippocampus after pilocarpine-induced SE. Photomicrographs showing the levels of astrocyte (anti-GFAP, A–C) and microglial cell (anti-ED1, E–H) activities in the normal (A), SE (B) and SE + HUMSC (C) groups at 29 days after SE. (D) Bar graphs showing the quantified mean value of astrocyte activities in the dorsal hippocampus in the normal, SE and SE + HUMSC groups. Pilocarpine-induced SE elevated significantly astrocyte activities in CA1, CA3, and the dentate hilus in the SE group (B). Astrocyte activity was prevented by HUMSCs transplantation (C). ED1-positive microglial cells were fewer in the SE + HUMSC group (g and h, arrows) than those in the SE group (E and F, arrows). * < 0.05, compared to normal rats. $^{\#}p < 0.05$, comparison between SE and SE + HUMSC groups. Scale bars = 500 μm (A–C); 100 μm (in CA1, CA3, and DH); 10 μm (E–H).

(Fig. 5A and B). However, such sprouting was decreased in the SE + HUMSCs rats, indicating that HUMSC transplantation significantly suppressed mossy fiber sprouting after pilocarpine-induced SE (Fig. 5C).

3.7. Survival and distribution of transplanted HUMSCs in the hippocampus

To track the migration of the transplanted HUMSCs, their nuclei were labeled with bisbenzimide. Moreover, anti-human specific nuclei antigen immunostaining was preformed to detect the engrafted HUMSCs. Bisbenzimide-labeled HUMSCs survived, and migrated along the rostrocaudal axis from the implantation site 29 days after transplantation. Large numbers of HUMSCs were found around the implantation site (Fig. 6A and B). Moreover, immunostaining showed that most of the engrafted HUMSCs survived in the hippocampus, and remained near the injection sites four weeks after transplantation (Fig. 6C and D). The results indicated that the transplanted HUMSCs survived and migrated in the host hippocampi.

3.8. Transplanted HUMSCs remain undifferentiated in the host hippocampus

To further investigate whether HUMSCs had differentiated into neuronal cells and astrocytes in rat hippocampus, human NeuN and GFAP were detected using RT-PCR. Human glioma acted as a positive control. As shown in Fig. 7A, against the positive detection of the human brain tissue, neither human NeuN nor GFAP mRNAs was detectable in the normal, SE, and SE + HUMSCs groups. Our results indicate that HUMSCs did not differentiate into neurons or astrocytes.

3.9. Transplanted HUMSCs expressed several cytokines in the rat hippocampus

To further elucidate the underlying mechanisms of the beneficial effects of HUMSC transplantation, cytokine expressions in the hippocampal tissues were examined using human cytokine arrays. The results showed that significantly elevated expressions of several growth promoting factors were associated with HUMSCs

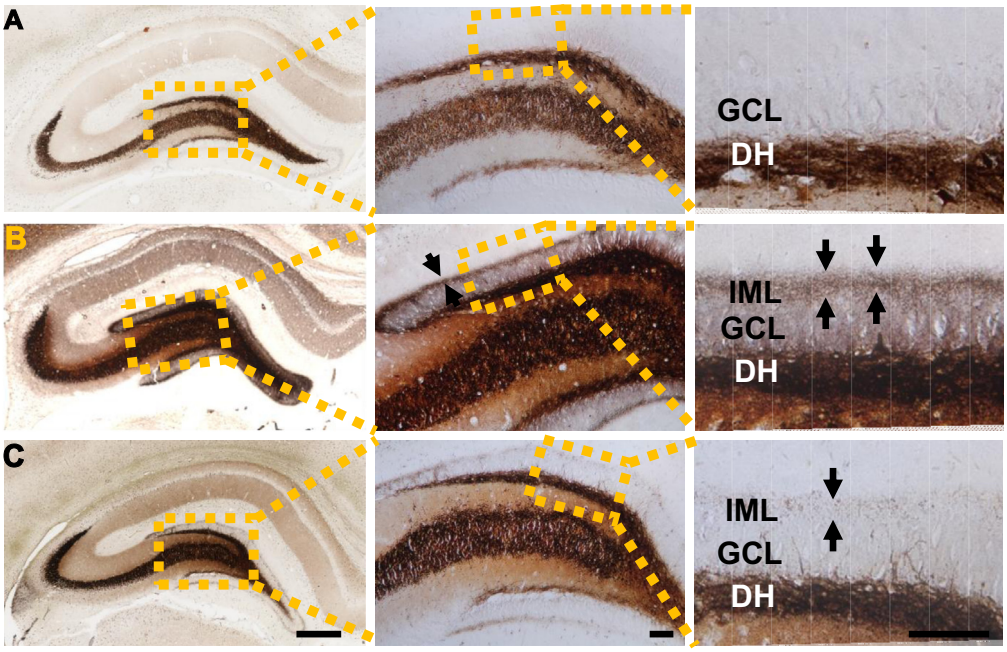

Fig. 5. HUMSC transplantation decreases mossy fiber sprouting in the hippocampus after pilocarpine-induced SE. Representative photomicrographs of Timm's staining showing mossy fiber sprouting in normal (A), SE (B), and SE + HUMSC (C) groups. Pilocarpine-induced SE led to marked aberrant mossy fiber sprouting in the inner molecular layer (arrows) of the dentate gyrus, which was attenuated by HUMSCs transplantation. The right panels are magnified images of the left boxed areas in A–C. Scale bars = 500 μm (A–C); 100 μm (enlarged panel).

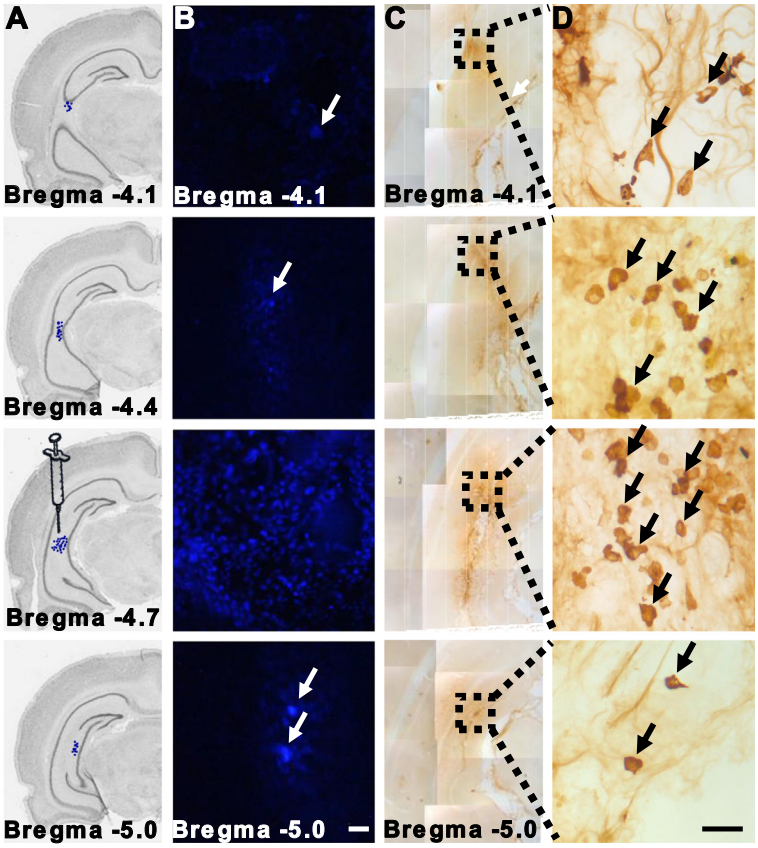

Fig. 6. Photomicrographs showing the survival and migration of grafted HUMSCs in the rat hippocampus. (A) Schematic drawings outline and (B) fluorescence photographs represent the location of bisbenzimide-labeled HUMSCs (blue dots) in the hippocampus at 29 days after SE at the bregma level. (C and D) Representative coronal sections show the nuclei of HUMSCs labeled using anti-human specific nuclear antigen in the hippocampus at the bregma level. HUMSCs survived in the hippocampus for 29 days after transplantation. The panels in D are magnified images of the left boxed areas in C. Arrows in D indicate positively stained cell bodies. Scale bar = 20 μm.

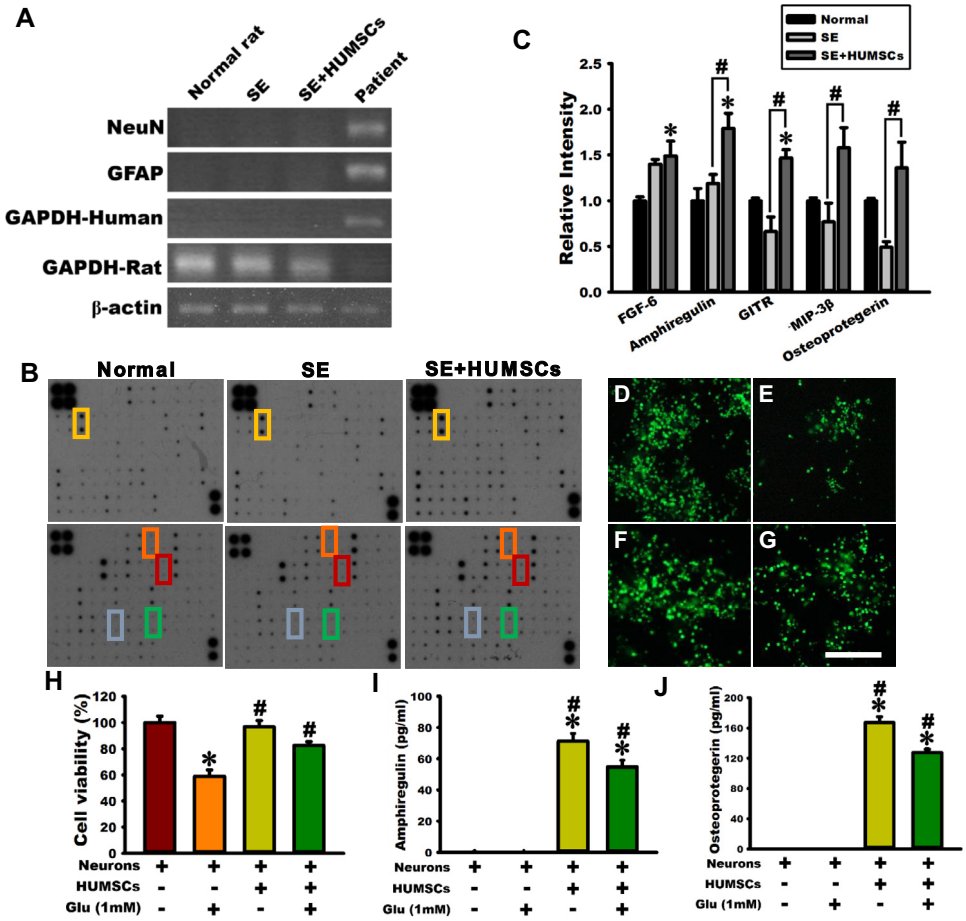

Fig. 7. HUMSCs released cytokines in the rat hippocampus and in the neuron and HUMSC co-cultures. (A) RT-PCR was performed to detect human NeuN and GFAP mRNA expression in rat hippocampus. Human NeuN and GFAP mRNAs were not detectable in the SE + HUMSC group. Human glioma cells were used as positive control, where NeuN and GFAP mRNAs were detected. (B and C) Several growth-promoting human cytokines are significantly increased in the SE + HUMSC group. Photomicrographs of the NeuroTrace fluorescent Nissl stain in cultured cortical neurons (D), neurons treated with glutamate (E), neuron and HUMSC co-cultures (F), and neuron and HUMSC co-cultures with glutamate treatment (G). (H) Histograms show that glutamate induced damage in the cortical neurons, and attenuation of glutamate-induced cytotoxicity by the HUMSC co-culture using an MTT assay. (I and J) Human osteoprotegerin and amphiregulin were detectable in the media of neuron and HUMSC co-cultures. Frames in blue: MIP-3β, red: GITR, orange: amphiregulin, green: osteoprotegerin, and yellow: FGF-6. * < 0.05, compared with the normal group; $^{\#}p < 0.05$, comparison between the SE group and SE + HUMSC group (C); * < 0.05, compared with the group of neurons without glutamate treatment; $^{\#}p < 0.05$ compared to the group of neurons with glutamate treatment (H–J).

transplantation. These growth-promoting factors included FGF-6, amphiregulin, glucocorticoid-induced tumor necrosis factors receptor (GITR), MIP-3β, and osteoprotegerin (Fig. 7B and C).

3.10. HUMSCs prevented glutamate-induced cytotoxicity in cultured cortical neurons

Using fluorescent NeuroTrace to label neuronal Nissl bodies, several cortical neurons were observed in the normal condition (Fig. 7D). However, the number of NeuroTrace-labeled cell bodies was reduced significantly after glutamate treatment (Fig. 7E). In transwell co-cultures of HUMSCs and neurons, there was no significant change in the number of cortical neurons after treatment with glutamate (Fig. 7F and G).

The results of an MTT assay revealed that glutamate decreased cell viability by 58.98 ± 4.84%, compared to that of neurons in the normal condition. However, the cytotoxic effects of the glutamate were attenuated by HUMSC co-culture ($p < 0.01$; Fig. 7H).

3.11. Human cytokines were detected in the medium of neurons and HUMSCs co-culture

To examine the protein levels of cytokine that were released by HUMSCs, the media from neurons alone or the neuron and HUMSC co-culture were subjected to human Quantikine ELISA analysis. Our analysis found abundant amphiregulin and osteoprotegerin in the medium of neuron and HUMSC co-cultures (Fig. 7I and J).

4. Discussion

In the present study, we investigated the potential therapeutic effects of HUMSCs in a rat pilocarpine-induced SE model. Transplanted HUMSCs in the acute phase of excitotoxic brain injury prevented tissue damage in the latent period and suppressed the development of SRMS.

Several types of cells, including embryonic stem cell and its derived cells, fetal hippocampal cells, neural stem cells, and bone

marrow mesenchymal stem cells have been investigated for the treatment of epilepsy in several experimental models (Acharya et al., 2008; Chu et al., 2004; Long et al., 2013; Shetty and Hattiangady, 2007). Boison did not find a seizure prevention effect for human embryonic stem cells in mice with an intra-amygdaloid injection of kainic acid (Boison, 2009). In a mouse model of TLE, Cunningham et al. demonstrated that engrafted hPSC-derived maturing GABAergic interneurons (mGINs) suppressed spontaneous seizure activity. The mGIN-grafted TLE mice showed a significantly reduced seizure event frequency. However, the seizure duration was not significantly different between control TLE mice and mGIN-grafted TLE mice (Cunningham et al., 2014). In our video/EEG recording analyses, rats transplanted with HUMSCs had a longer latency period for developing spontaneous seizure, a lower seizure frequency, and a shorter seizure duration. Moreover, seizure activity in all of the animals of the SE + HUMSCs group was absent entirely at the second and the fourth weeks. These findings indicate that transplantation of HUMSCs into bilateral hippocampi ameliorates seizure activity in the rat TLE model.

To determine the brain alterations after pilocarpine-induced SE, an MRI was performed. Brain edema in the hippocampus, amygdala and piriform cortex was most prominent approximately two days after SE, which is consistent with a previous finding of vasogenic edema due to BBB failure at two days post-SE (Itoh et al., 2015). In the present study, brain edema in the hippocampus, the lateral ventricle, the 3rd ventricle, thalamus and piriform cortex persisted for up to 29 days in the SE group. The temporal profile is in agreement with the morphological and volume changes in the dorsal hippocampus after SE. Notably, our results further showed that HUMSC transplantation did delay and attenuate the development of SE-induced brain edema.

TLE is characterized by neuronal loss and gliosis in the hippocampus (Borges et al., 2003; do Nascimento et al., 2012). A previous study used Fluoro-Jade to analyze the temporal pattern of neuronal damage, and GFAP immunohistochemistry to investigate gliosis occurring in the hippocampal formation of mice at 3, 6, 12, and 24 h, and one and three weeks after the pilocarpine-induced status-epilepticus (SE) onset. The results showed that the maximum neuronal damage in the hilus of the dentate gyrus appeared at 3 h after SE onset, and neuronal injury in the CA1 and CA3 pyramidal cell appeared at one week after SE onset. The highest presence of GFAP immunoreaction in CA1 and CA3 was expressed at one and three weeks after SE onset, respectively (do Nascimento et al., 2012). Many studies have examined the effects of SRMS and multiple epileptogenic changes in chronically injured hippocampus, including aberrant mossy fibers sprouting (Okazaki et al., 1995; Shetty and Turner, 1997; Shetty et al., 2003), the loss of calbindin (Shetty and Turner, 1995), and reductions in the number of GABAergic interneurons (Esclapez et al., 1997; Franck et al., 1988; Mathern et al., 1995; Shetty and Turner, 2000; Sloviter and Nilaver, 1987). In our study, brain edema reached a plateau at 15 days after pilocarpine treatment and persisted up to 29 days in the SE group. In agreement with the evidence from MRI images, Nissl staining results showed that the morphology of the dorsal hippocampus was abnormal, and the dorsal hippocampal volume in the SE group was significantly decreased. Moreover, quantitative calculation of pyramidal neurons and GABAergic neurons in the hippocampus demonstrated that the number of pyramidal neuron and GABAergic neurons was decreased significantly in the CA1 and CA3 regions in the SE group at Day 29 after SE. In our present study, the extent of the SE-induced loss of hippocampal pyramidal and GABAergic neurons was significantly reduced by HUMSCs transplantation. The results suggest that the suppression of SRMS might underlie the reduction in brain edema, preservation of pyramidal cells and GABAergic inhibitory interneuron circuitry.

GFAP is a risk marker of acute injury and astrogliosis (Gurnett et al., 2003). Several authors have suggested that astrocytes might have an important role in the generation and spread of seizure activity (Hammer et al., 2008; Seifert and Steinhauser, 2013). The reactive astrocytes during the process of epileptogenesis might be associated with the release of trophic factors that lead to axonal sprouting, synapse formation, and hyper-excitability (O'Dell et al., 2012). In the present study, GFAP positive cells were increased in CA1, CA3 and dentate gyrus. This result provides evidence of possible positive functions of grafted HUMSCs in a TLE animal model. HUMSCs might inhibit SE-induced brain inflammation.

Lee and coworkers found that the frequency and the duration of spontaneous recurrent motor seizures in pilocarpine-induced animals were reduced by the transplantation of human neural stem/progenitor cells (NSPCs) into the hippocampus (Lee et al., 2014). The effect was likely due to the differentiation of human NSPCs into GABAergic interneurons. Bone marrow mesenchymal stem cells (BMSCs) or genetically engineered BMSCs were injected intravenously, transplanted intra-ventricularly, or implanted directly into the hippocampus to evaluate the recovery of pilocarpine-induced epileptic rats. The results showed that the number of seizures significantly decreased, and the density of neurons per area was higher in the transplanted group (Abdanipour et al., 2011; Costa-Ferro et al., 2012, 2010; Huicong et al., 2013; Leal et al., 2014; Li et al., 2009; Long et al., 2013). Regarding human cord blood, transplantation of HUCBC into pilocarpine-induced epileptic rats protected against neuronal loss in the hippocampal subfields CA1, CA3, and in the hilus of the dentate gyrus. Moreover, the frequency and duration of spontaneous recurrent seizures after SE were reduced in transplanted rats (Leal et al., 2014).

In addition to neuronal protection, therapeutic stem cell transplantation also improves neurogenesis (Shetty, 2012). A previous study showed that chronic TLE was associated with a severe decrease of neurogenesis in the adult hippocampus (Hattiangady et al., 2004). Other studies showed that the implantation of neuronal stem cells or progenitors into the hippocampus of aged rats stimulated neurogenesis from endogenous neuronal stem cells in the dentate gyrus (Hattiangady et al., 2007; Park et al., 2010). These results reveal that stem cell transplantation to improve the neurogenesis may become a promising therapeutic approach. Most likely, the cytokine release from stem cells critically influences neurogenesis. In our present study, the SE-induced loss of hippocampal pyramidal and GABAergic neurons was significantly reduced by HUMSCs transplantation. Our results indicate that HUMSC implantation may provide supplemental neuronal protection and stimulate neurogenesis.

Ideal donor cells for neurological disease therapy should have the following characteristics: (i) be easily available; (ii) be capable of rapid expansion in culture; (iii) be immunologically compatible; (iv) be capable of long-term survival and integration in the host tissue, and (v) be amenable to stable transfection and long-term expression of exogenous genes (Bjorklund, 1993). HUMSCs in Wharton's jelly of the umbilical cord can be easily obtained and processed compared to embryonic or bone marrow stem cells. Approximately 1×10^6 HUMSCs were collected from a 20-cm umbilical cord. The number of HUMSCs doubled (2×10^6) in 10% fetal bovine serum (FBS)-Dulbecco's modified Eagle's medium (DMEM) in three days. Our previous studies have shown that the transformed HUMSCs in the striatum were still viable four months after transplantation without the need for immunological suppression (Fu et al., 2006). Moreover, transformed HUMSCs survived in the rat liver and were able to control Type 1 Diabetes (Chao et al., 2008). Likewise, the HUMSCs in the present study survived in rat spinal cords, suggesting that HUMSCs are an ideal stem cell source for transplantation.

Interestingly, HUMSCs express different cytokines when engrafted into fibrotic liver, infarct cortex or the injured spinal cord depending on the different pathological microenvironments (Lin et al., 2011; Tsai et al., 2009; Yang et al., 2008). We previously showed that transplantation of HUMSCs could serve as a therapeutic option for ischemic stroke. Implanted HUMSCs released neuroprotective and growth-associated cytokines including neutrophil-activating protein-2 (NAP-2), angiopoietin-2, brain-derived neurotrophic factor (BDNF), CXCL-16 and platelet-derived growth factor-AA (PDGF-AA). These cytokines can promote endogenous neurogenesis, protect neurons from damage, and improve motor function after ischemic stroke, and therefore contribute to the overall therapeutic effects of HUMSC transplantation (Lin et al., 2011). Interestingly, HUMSCs release different cytokines according to the different organs in which they are grafted. When HUMSCs are implanted into the rat spinal cord after complete transection, instead of differentiating into neurons, astrocytes, or oligodendrocytes, they release human cytokines and growth factors such as neurotrophin-3, NAP-2, basic fibroblast growth factor (bFGF), GITR, and vascular endothelial growth factor receptor 3 (VEGF-R3), which may be involved in the repair and regeneration of the spinal cord (Yang et al., 2008). Furthermore, without differentiating into hepatocytes, HUMSCs grafted into rat fibrotic liver release a considerable amount of human prolactin, leukemia inhibitory factor (LIF), and cutaneous T cell-attracting chemokine (CTACK) (Tsai et al., 2009). Therefore, depending on the pathophysiological microenvironment, it is likely that HUMSCs release different cytokines and respond accordingly.

The expression of 174 human cytokines was measured in this study, including pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α (De Simoni et al., 2000; Grall et al., 2003). We found that the amount of human pro-inflammatory cytokines did not change in the SE + HUMSC group relative to those of the normal and SE groups. Nevertheless, five human cytokines or growth factors were increased in the hippocampi of the SE + HUMSC group, which were FGF-6, amphiregulin, GITR, MIP-3β, and osteoprotegerin. These cytokines may execute various functions and play distinct roles. Above all, MIP-3β may enhance the production of anti-inflammatory cytokine IL-10 by activated human peripheral blood monocytes and T cells, suggesting that MIP-3β could participate in anti-inflammatory activity (Byrnes et al., 1999). Extracellular-signal-regulated kinase (ERK) phosphorylation and NANOG are critically involved with self-renewal, proliferation, and undifferentiation in pluripotent embryonic stem cells (Chen et al., 2012; Mitsui et al., 2003). FGF-6 treatment could sustain *NANOG* expression levels and support ERK phosphorylation in ESCs (Chen et al., 2012). Moreover, MIP-3β could induce ERK phosphorylation in a dose-dependent fashion (Yu et al., 2015). In this study, human FGF-6 and MIP-3β were up-regulated in the SE + HUMSC group, suggesting that FGF-6 and MIP-3β expression in HUMSCs in the host hippocampi may help to maintain their undifferentiated state and release of cytokines. This observation was consistent with the RT-PCR results, neither human NeuN nor GFAP mRNAs were detectable in the SE + HUMSC group, indicating that HUMSCs did not differentiate into neurons or astrocytes. Previous studies have shown that MIP-3β could promote cell proliferation, cell invasion, and cell migration (Peng et al., 2015; Yu et al., 2015). Amphiregulin stimulates axonal outgrowth, and act as a neuronal survival factor (Falk and Frisen, 2002; Nilsson and Kanje, 2005). GITR is important for neuronal development. Activation of GITR could inhibit the nuclear factor-kappaB signal pathway, and regulate the extent of sympathetic neurite growth (McKelvey et al., 2012). Osteoprotegerin plays a neuroprotection role in the cochlear nerve (Kao et al., 2013). From our results, human cytokines released from HUMSCs might be involved in processes of neuroprotection and anti-inflammation after transplantation into pilocarpine-induced epilepsy rats. Our *in vitro* results support a similar conclusion. Our results demonstrate that treatment of cultured cortical neurons with glutamate caused neuronal damage; however, co-culturing with HUMSCs might prevent such neuronal loss. Furthermore, large quantities of amphiregulin and osteoprotegerin were present in the medium of the neuron and HUMSC co-cultures.

5. Conclusion

Our study shows that intra-hippocampal transplantation of HUMSCs can suppress the spontaneous recurrent seizures in a pilocarpine TLE model. The underlying mechanisms of the early therapeutic effects include a decrease of neuron and interneuron loss, suppression of SE-induced brain inflammation, and a reduction of MFS. Our results suggest that cytokines released from transplanted HUMSCs are neuroprotective against epileptogenesis.

Conflict of interest

The authors indicate no potential conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2015.12.021.

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