Mesenchymal stem cells inhibit transmission of α-synuclein by modulating clathrin-mediated endocytosis in parkinsonian models

Ha Na Kim

Division of Medical Science

The Graduate School, Yonsei University

Mesenchymal stem cells inhibit transmission of α-synuclein by modulating clathrin-mediated endocytosis in parkinsonian models

Directed by Professor Phil Hyu Lee

The Master's Thesis

submitted to the Division of Medical Science,

the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Master of Medical Science

Ha Na Kim

June 2015

This certifies that the Master's Thesis

of Ha Na Kim is approved.

Thesis Supervisor: Phil Hyu Lee

Thesis Committee Member #1: Sung-Rae Cho

Thesis Committee Member #2: Ki Taek Nam

The Graduate School

Yonsei University

June 2015

ACKNOWLEDGEMENTS

논문을 완성하기 까지 도움을 주신 많은 분들께 감사 드립니다. 혼자서는 해낼 수 없었던 일을 가능하게 해주신 이필휴 교수님께 깊은 감사의 말씀 드립니다. 연구주제를 받았을 때 파킨슨병에 관한 연구를 한다는 생각에 기뻤습니다. 연구를 하는 내내 흥미로웠고 결과가 나올 때 마다 행복했습니다. 즐겁게 공부할 수 있도록 항상 연구에 열정을 불어넣어주신 교수님께 다시 한번 감사 드립니다. 교수님의 모습을 보면서 제 자신을 반성하기도 하고 더 열심히 하고자 노력했던 것 같습니다. 정말 존경합니다. 실험과 연구방법을 지도해주시고 실험이 재미있는지 힘든 건 없는지 물어 봐주시고 신경 써주신 박현정선생님과 논문의 전반적인 계획과 실험 방법을 지도해주시고 제가 올바른 선택을 할 수 있도록 조언해주신 신진영선생님께 감사의 말씀 드립니다. 3년이라는 시간 동안 연구실 생활을 하며 성장할 수 있었던 시기였고 기회였습니다. 앞으로 다른 곳에서도 더 발전해나가도록 하겠습니다.

김하나 올림

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Mesenchymal stem cells inhibit transmission of α-synuclein by modulating clathrin-mediated endocytosis in parkinsonian models

Ha Na Kim

Division of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Phil Hyu Lee)

Ample evidence has suggested that misfolded α-synuclein can be released from cells and transmitted from one brain area to others through cell-to-cell propagation in Parkinson's disease (PD). In terms of prion-like behavior, extracellular α-synuclein plays key roles in the pathogenesis and progression of α-synucleinopathies. Mesenchymal stem cells (MSCs) secrete various cytotropic factors that have neuroprotective effects through complex mechanisms, such as modulation of neuroinflammation, enhancement of cell survival signals, increased neurogenesis, and modulation of autophagy. In the present study, we investigated whether MSCs could exert neuroprotective effects through modulation of cell-to-cell transmission on extracellular α-synuclein. Using α-synuclein-enriched models, we showed that mesenchymal stem cells (MSCs) inhibited cell-to-cell transmission by blocking the clathrin-mediated endocytosis of extracellular α-synuclein via modulation of the interaction with N-methyl-D-aspartate receptors, which led to a prosurvival effect on neurons with functional improvement of motor deficits. Moreover, MSC treatment significantly inhibited transmission of α-synuclein from ipsilateral to contralateral hemisphere of α-synuclein inoculation compared to α-synuclein treated PD animals. Furthermore, Galectin-1, soluble factors derived from MSCs, played an

important role in the transmission control of extracellular α-synuclein in these models. Our data suggest that MSCs exert neuroprotective properties through inhibition of cell-to-cell transmission of extracellular α-synuclein, which may be applicable to clinical strategies for treatment of PD patients.

Key words: mesenchymal stem cell, α-synuclein, transmission, parkinson's disease

Mesenchymal stem cells inhibit transmission of α-synuclein by modulating clathrin-mediated endocytosis in parkinsonian models

Ha Na Kim

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Phil Hyu Lee)

I. INTRODUCTION

α-Synuclein consists of 140 amino acids and is found naturally as an unfolded cytoplasmic protein in neuronal synaptic terminals. However, overexpression of α-synuclein interrupts normal cell functions and leads to decreases in neurite outgrowth and cell adhesion.[1] α-Synuclein aggregates comprised of monomeric, oligomeric intermediate, or fibrillar forms are thought to be involved a critical step in the pathogenesis of Parkinson's disease (PD) and in other α-synucleinopathies, such as multiple system atrophy and dementia with Lewy bodies.[2]

Oligomeric and monomeric α-synuclein have both been detected in cerebrospinal fluid and plasma samples from PD patients, suggesting that small aggregates of α-synuclein access the extracellular space.[3-5] Previous animal and clinical data suggest that misfolded α-synuclein can be released from cells by exocytosis and transmitted from one brain area to another via cell-to-cell propagation.[6,7] Although the exact mechanism of α-synuclein transmission remains unknown, evidence suggests that clathrin-mediated endocytosis (CME) may have an important role in internalization of extracellular α-synuclein.[8,9] As the cargo protein for endocytosis is usually recognized by a specific receptor on the

cell surface,[10-12] it is possible that α-synuclein may interact with cell-surface receptors that have not been well specified until now. N-methyl-D-aspartate (NMDA) receptor subunits contain motifs that bind the endocytic adaptor protein involved in CME,[13] and a recent study provided the evidence that α-synuclein participates in CME through interaction with an NMDA receptor.[9] Accordingly, α-synuclein propagation from one area of the brain to others via cell-to-cell transmission is closely related with disease progression or clinical severity. Thus, strategies targeting modulation of α-synuclein transmission be important for development of future disease modifying therapies in individuals with α-synucleinopathies.

Mesenchymal stem cells (MSCs) secrete various cytotropic factors including neurotrophic growth factors, chemokines, cytokines, and extracellular matrix protein, which in turn, exert neuroprotective effects.[14-16] In previous studies, we showed that MSCs have potent neuroprotective effects through modulation of neuroinflammation, inhibition of apoptotic cell death, increases in neurogenesis and neuronal differentiation, and enhancement of autophagy in neurodegenerative models.[17-21] In the present study, we evaluated whether MSCs would inhibit cell-to-cell transmission of extracellular α-synuclein and thus exert a neuroprotective effect using α-synuclein enriched models. Furthermore, we determined that galectin-1 (Gal-1), the biological molecule secreted from MSCs, plays a crucial role in modulation of extracellular α-synuclein transmission.

II. MATERIALS AND METHODS

1. α-Synuclein aggregate preparation and fluorescent dye labeling

Recombinant α-synuclein (200 μM in phosphate buffered saline (PBS; HyClone, Irvine, CA,USA)) was agitated at 37 ˚C (250 rpm) for 14 days. After brief sonication, the protein was incubated for another 7 days. Aggregated protein was collected by ultracentrifugation at 200,000 × *g* for 1 hr, and the pellet was resuspended in PBS with brief sonication. Alexa Fluor 488 labeling of α-synuclein aggregate was performed according to the manufacturer's instructions (Invitrogen, Carlsbad, CA,

USA). Briefly, aggregate proteins were incubated with 24-fold molar excess of Alexa Fluor 488 at room temperature for 1 hr. Excess unbound Alexa Fluor 488 dye was removed by passing through a desalting column.

2. MSCs and SH-SY5Y culture

Frozen vials of characterized human MSCs at passage 2 were obtained from the Severance Hospital Cell Therapy Center (Seoul, South Korea). The human neuroblastoma cell line, SH-SY5Y cells were obtained from the Korean Cell Line Bank (Seoul, South Korea). Both MSCs and SH-SY5Y cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; HyClone) supplemented with 10% fetal bovine serum (HyClone) and an antibiotic mixture of penicillin and streptomycin (1%, HyClone). When these cells reached 70–80% confluence, they were trypsinized and subcultured. These cells were cultivated in a humidified incubator at 37 ˚C and 5% CO_2 before use. For *in vitro* experiments, SH-SY5Y was plated at a density (of $1.5\times10^4/cm^2$) and treated with CM. For differentiation, SH-SY5Y was plated at a density (of $5\times10^5/ cm^2$) and grown as monolayer in DMEM. 1 day after plating, the cells were incubated in fresh DMEM with 10 μM retinoic acid (Sigma, St. Louis, MO, USA). The medium was changed on alternate days, and cultures were allowed to differentiate for 2 wk[22] and then treated with α-synuclein (1 μM) or Gal-1 (100 ng) for 2 hr. Additionally, the effects of MSCs were tested in differentiated SH-SY5Y that was co-cultured without direct contact using a Costar transwell (Corning, Big Flats, NY, USA). The MSCs were cultured on the permeable membrane of Costar transwell insert and the differentiated SH-SY5Y cells were maintained on the bottom of a plate. For inhibition of α-synuclein clearance, Bafilomycin A1 (50 nM, Sigma) was added to the medium containing α-synuclein. For inhibition of endocytosis, Dynasore (80 μM, Sigma) was pre-treated to the replacement medium for 2 hr. For inhibition of endocytosis, MK-801 hydrogen maleate (50 μM, Sigma) was pre-treated to the replacement medium for 3 hr. All experiments were replicated 3 times.

3. Plasmid Transfections

Transfection was performed using Superfect (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. Differentiated SH-SY5Y cells were maintained in OPTI-MEM medium

supplemented with 10% FBS and incubated at 37 ˚C. Cells were plated 24 hr prior to transfection, growing to 80–90% confluence prior to transfection. The cells were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. For culture of donor and acceptor cells, the acceptor cells were transfected with mCherry-tagged (Red) endosomal Rab5. The donor cells were prepared by incubating with Alexa Fluor 488 labeling of α-synuclein for 3 hr. After PBS washing and trypsinization, the donor cells (40,000 cells per dish mixed (1:1)) were co-cultured for 24 hr on the top of the acceptor cells.

4. Preparation of cell CM

CM were prepared as follows: 80% confluent MSCs at passage 5 and SH-SY5Y cells were fed with serum-free DMEM. The medium of MSCs and SH-SY5Y cells were both assumed to contain various paracrine molecules. These medium were collected.

5. 2D-PAGE

2D-PAGE was basically performed as described previously.[23] Briefly, 200 μg of protein extract were separated by isoelectric focusing using an IPG strip with a nonlinear pH gradient of 4–10 for the first dimension, and then sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 26×20-cm format) for the second dimension. Proteins were detected by alkaline silver staining as described previously.[24] Image analysis and quantification of protein spots were performed using the PDQuest software (Bio Rad, Hercules, CA, USA). The quantity of protein in each spot was normalized relative to the total valid spot intensity.

6. MALDI-TOF/MS analysis

For PMF, protein spots were excised, digested with trypsin (Promega, Madison, Wisconsin, USA), mixed with α cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid, and subjected to MALDI-TOF/MS analysis by Microflex LRF 20 (Bruker Daltonics, Billerica, MA, USA) as described.[25] Spectra were collected from 300 shots per spectrum over the m/z range 600–3000 and calibrated by two-point internal calibration using trypsin autodigestion peaks (m/z 842.5099,

2211.1046). The peak list was generated using Flex Analysis 3.0 (Bruker Daltonics). The thresholds used for peak-picking were as follows: 5000 for minimum resolution of monoisotopic mass, 2.5 for *S*/*N*. The profound (http://prowl.rockefeller.edu/prowl-cgi/profound.exe) program was used to search the human NCBInr database for protein identification. The following parameters were used for the database search: trypsin as the cleaving enzyme, a maximum of one missed cleavage, iodoacetamide as a complete modification, oxidation as a partial modification, monoisotopic masses, and a mass tolerance of ± 0.1 Da. PMF acceptance criteria were based on probability scoring.

7. Cell viability analysis

SH-SY5Y cells were harvested and plated in 96-well polystyrene plates (Corning) at a 1.5×10^4 cells per 100 μL of medium per well. Plates were incubated at 37 ˚C for 24 hr to allow cells to attach. After 24 hr, the medium was exchanged with 100 μL of the preincubated mixtures of α-synuclein with CM or SH-SY5Y cells were directly treated with α-synuclein. The same volume of DMEM was added to the control cultures. Plates were then incubated at 37 ˚C for an additional 24 and 48 hr. Cell viability was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assays essentially as described.[26] Briefly, after the cells were incubated with the various medium samples, MTT was added to a final concentration of 0.5 mg/mL. After incubation at 37 ˚C for 3 hr, the plats were centrifuged and the medium was aspirated from each well. The absorbance was measured by an ELISA microplate reader (VersaMax, Sunnyvale, CA, USA) at 490 nm. Cell viability was calculated by dividing the absorbance of wells containing samples (corrected for background) by the absorbance of wells containing medium alone (corrected for background).

8. Reverse transcription-polymerase chain reaction (RT-PCR)

To knock down Gal-1 in MSCs cells, Gal-1 small interfering RNA (siRNA) construct (Santa Cruz, Santa Cruz, CA, USA) was purchased and tested for knockdown efficiency. MSCs were plated at a density of $1\times10^5/cm^2$ and incubated with 8 μL of 20 μM siRNA (final concentration 40 nM) in 100 μL of Opti-Minimum Essential Media (Gibco, Grand Island, NY, USA) containing 10 μL of Lipofectamine 2000 (Invitrogen). After 72 hr, total RNA was extracted from the MSCs using TRIzol®

reagent (Invitrogen) in accordance with the manufacturer's protocol. An equal amount of RNA (1 μg) in each experiment was reverse transcribed using amfiRivert cDNA Synthesis Premix (GenDEPOT, Barker, TX, USA). Subsequently, 2 μL of cDNA were used as a template for RT- PCR analysis in amfiRivert 1-Step RT-PCR Kit (GenDEPOT). PCR was performed using 10 pmol of primers for human Gal-1(Forward 5'- GCAACCTGAATCTCAAACC -3', Reverse 5'- GGCCACACATTTGATCTTG -3'). After an initial denaturation at 95 ˚C for 2 min, 30 cycles of PCR were performed, consisting of denaturation (1 min, 95 ˚C), annealing (1 min, 49.8 ˚C) and extension (1 min, 72 ˚C) followed by a final extension step (5 min, 72 ˚C). The PCR products were separated by electrophoresis on 2% agarose gels (Intron, Seongnam-si, Kyungki-do, South Korea) and stained with ethidium bromide (Sigma). Gels were examined under UV illumination (Bio Imaging Systems, Jerusalem, Israel). Density was measured using the Image Gauge v.4.0 software (Fujifilm Science Laboratory, Tokyo, Japan).

9. Animal study

All procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent Experiment provided by the Institutional Animal Care and Use Committee (IACUC) at the Yonsei University Health System. Male C57BL/6 mice (Orient Bio, Seongnam-si, Kyungki-do, South Korea) were acclimated in a climate-controlled room with a constant 12 hr light/dark cycle for 1 week prior to the initiation of drug administration. At 6 weeks of age, the mice received α-synuclein (5 μg/mouse) with and without dynasore (80 μM/mouse) was administered via the neocortex. Cortical administration was carried out in accordance with the procedure described previously with minor modifications.[7] Briefly, mice were anesthetized with isoflurane (Baxter, Deerfield, IL, USA), and slowly injected bilaterally into the cortex (0.4 mm posterior to bregma, -1.3 mm lateral to midline and -0.6 mm ventral to the brain surface) using a stainless-steel injection needle (26-gauge) connected to a 10 μL Hamilton microsyringe (Hamilton, Reno, NV, USA). The needle was left in place for 10 min before being withdrawn slowly. To evaluate the short-term effects of MSCs on α-synuclein transmission, the mice were randomly divided into three groups (n = 5 per group): (1) Control; (2) α-synuclein; (3) α-

synuclein and MSC. Control mice were injected with saline via tail vein at 1 day after α-synuclein inoculation (postoperative day 1). Mice in the MSC group were subjected to MSCs into the tail vain (1×10^6 cells /200 μL) for stereotactic injection at the same time. All mice were sacrificed on post-operative day 7. Additionally, to evaluate the modulation of α-synuclein transmission by MSC-CM or Gal-1, the mice were randomly divided into four groups (n=5 per group): (1) Control; (2) Fresh medium; (3) MSC-CM; (4) Gal-1. Mice in the each group were subjected to medium delivery on postoperative day 1, and all mice were sacrificed on postoperative day 7. Finally, to evaluate the long-term effects of MSCs on α-synuclein transmission, the mice were randomly divided into three groups (n = 5 per group): (1) Control; (2) α-synuclein; (3) α-synuclein and MSC. α-Synuclein was slowly injected bilaterally into the striatum (0.2 mm posterior to bregma, ±2.0 mm lateral to midline, and -2.6 mm ventral to the brain surface). Mice in the MSC group were subjected to MSCs into the tail vain (1×10^6 cells /200 μL) on postoperative day 1 and postoperative day 10. All mice were sacrificed on postoperative day 30.

10. Brain sample preparation

For immunochemical analysis, all mice were deeply anesthetized with chloral hydrate (I.P., 0.4 g/kg; Fluka, Steinheim, Germany) and then perfused with 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (pH 7.4). The brains were embedded in paraffin, and coronal sections 4 μm thick were then cut and placed on slides.

11. Immunocytochemistry and immunohistochemistry

SH-SY5Y cells and brain sections on deparaffinized tissue sections were washed twice in PBS and incubated in 0.2% Triton X-100 (Sigma) for 30 min at room temperature. They were blocked with 0.5% bovine serum albumin (BSA; Sigma) for 30 min. After blocking, they were rinsed three times with 0.5% BSA and incubated overnight at 4 °C with specific primary antibodies. The primary antibodies used as follows: mouse anti-α-synuclein (Millipore, Billerica, MA, USA), rabbit anti-α-synuclein (phospho S129, Abcam, Cambridge, UK), rabbit anti-EEA1 (Abcam), mouse anti-Clathrin (Sigma), mouse anti-NuMA (Millipore), rabbit anti-Gal-1 (Abcam) and mouse anti-TH (Sigma). For detection of surface

NR1 and NR2A subunits, the cells and tissues were directly treated overnight at 4 ˚C with mouse anti-NR1 (Abcam) and rabbit anti-NR2A (Millipore) without permeabilization with Triton X-100. Immunofluorescence labeling was carried out by incubating the cells with rabbit anti-IgG Alexa Fluor-555 (Invitrogen), mouse anti-IgG Cy-3 (Chemicon, Temecula, CA, USA), rabbit anti-IgG Cy-3 (Chemicon) and rabbit anti-IgG FITC (AP132F, green; Chemicon). The cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen). The TH antibodies and phosphorylated α-synuclein antibodies were detected with 0.05 % diaminobenzidine (DAB, Vector Laboratories, Burlingame, CA, USA). The immunostained cells were analyzed using bright-field microscopy and viewed under a Zeiss LSM 700 confocal imaging system (Zeiss, Heidelberg, Germany). To analyze the localizations of antigens in double-stained samples, immunofluorescence images were created from the same tissue sections and merged using the Zeiss ZEN software (Zeiss).

12. Western blotting analysis

To extract membrane protein, we used Qproteome cell compartment kit (QIAGEN, Redwood City, CA, USA) following the manufacturer`s instructions. Cells and brain tissues were dissolved in ice-cold RIPA buffer (50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) (Sigma) plus protease inhibitor cocktail (Sigma). The lysates were centrifuged at 4 ˚C for 20 min (14,000 × *g*) and supernatants were transferred to fresh tubes. Briefly, 50 μg and 100 μg of protein were separated by SDS-gel electrophoresis and transferred onto hydrophobic PVDF membranes (GE Healthcare, Buckinghamshire, UK). The membranes were blocked in nonfat milk (BD, Franklin Lakes, New Jersey, USA). Membranes were probed with the following primary antibodies: rabbit anti-α-synuclein (Millipore), rabbit anti-α-synuclein (phospho S129, Abcam), rabbit anti-EEA1 (Abcam), mouse anti-Clathrin (Sigma), mouse anti-NR1 (Abcam), rabbit anti-NR2A (Millipore), rabbit anti-Gal-1 (Abcam), rabbit anti-caspase-3 (Cell Signaling, Danvers, MA, USA), mouse anti-actin (Santa Cruz), and rabbit anti-α-tubulin (Santa Cruz). As secondary antibodies, a 1:10000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody (GenDEPOT) and anti-mouse antibody (GenDEPOT) were used. Antigen–antibody complexes were visualized with a chemiluminescence system (Santa cruz),

followed by exposure to X-ray film (Fujifilm). For semiquantitative analysis, immunoblotting band densities were measured by computer imaging.

13. TUNEL Assay

Fragmented DNA was detected in apoptotic cells by adding Fluorescein 12-dUTP to nicked ends of DNA (In Situ Cell Death Detection Kit, Roche, Basel, Schweiz). Slides were incubated for 1 hr at 37 °C in the dark, followed by a wash with PBS three times and stained with DAPI. Red fluorescence was correlated with DNA fragmentation. For quantitative measure of apoptosis, it was defined as a percentage of apoptotic cells per total number of cells. For the purpose of this study, we used the following terminology: TUNEL labelling index (LI) = Number of TUNEL-positive cells × 100 / Total number of nuclei. We selected eight random 200× fields per sample for all indices and counted approximately 1,000 cells for each sample.

14. Rotarod test

To assess motor function and coordination, and balance, mice were tested on the Rotarod apparatus (MED-Associates). On the day before the training session started, mice were habituated to the apparatus for 15 min. In training trials, mice were trained to run on the rotarod (20 rpm) for 10 min without falling, twice a day for three consecutive days before α-synuclein administration. In test trials, mice were placed on the rotarod with increasing speed, from 4 rpm to 40 rpm and were placed on the rotarod at 30 rpm (cut-off time 700 s maximum). The latency time to fall was recorded.

15. Pole test

The pole test was performed according to a previous study.[27] Each mouse was placed on the top of a vertical wooden rough-surfaced pole (1 cm in diameter and 50 cm in height). On the day prior to testing, mice were habituated to the apparatus by placing them at the top of the pole and allowing them to descend five times. The total time that it took each mouse to reach the base of the pole and place all four paws on the floor was recorded. For each session of five descents, the best performance was recorded as the total time. If the mouse was unable to turn completely downward, fell off, or

slipped down the pole, a default value of 120s was recorded.

16. Measurement of α-synuclein

The amount of a-Synuclein was measured using sandwich ELISA kit (AnaSpec, San Jose, CA, USA). 10 μL of each diluted samples, and standards included in the kit were applied to microtiter plates precoated with antibody that specifically recognized a-Synuclein. Following an overnight incubation at 4 ˚C and washing, a detection antibody indirectly linked to an enzyme was applied. After incubation and washing, 350 μL of wash solutions were added to each well and then invert plate dry by hitting plate until no moisture appears. The substrate was added and incubated for 15 min at 37 ˚C, and then the reaction was stopped with stop solution. The color reaction was measured with an automatic ELISA microplate reader (BIOTECH, Winooski, VT, USA) with the wavelength set at 450 nm. The software (Bio Rad) was used to create standard curves and to calculate the concentration of the samples.

17. Stereological cell counts

TH-stained neurons were counted in the right and left SN pars compacta (SNpc) of every fourth section throughout the entire extent of the SNpc. Each midbrain section was viewed at low power at a random start and then the number of TH-stained cells was counted at high power. To avoid double counting of neurons with unusual shapes, TH-stained cells were counted only when their nuclei were optimally visualized, which occurred only in one focal plane. After all of the TH-stained neurons were counted, the total numbers of TH-stained neurons in the SNpc were calculated by using the formula described.[28]

18. Statistical analysis

The group means were compared using the Mann-Whitney U-test for pairs and the Kruskal-Wallis analysis for multiple groups. *P* values less than 0.05 were considered statistically significant. Statistical analyses were performed using commercially available software (version 12.0; SPSS Inc.).

III. RESULTS

1. MSCs inhibit internalization and cell-to-cell transmission of extracellular α-synuclein in neuronal cells

When α-synuclein fibrils labeled with Alexa 488 were incubated with SH-SY5Y cells, punctate and vesicular patterns of α-synuclein were revealed in the cytoplasm. Co-culture with MSCs (Figure 1A) markedly decreased internalization of labeled α-synuclein fibrils (Figure 2A); however, co-culture with neuronal cells had no modulatory effects on α-synuclein internalization (data not shown). A donor-acceptor co-culture method (Figure 1B) demonstrated that the transfer of α-synuclein from donor cells to connected acceptor cells occurred in the α-synuclein treatment group; however, transmission of α-synuclein from donor cells to acceptor cells was observed infrequently in the group of co-culture with MSCs (Figure 2B). Thus, co-culture with MSCs significantly decreased the levels of internalized cytosolic α-synuclein with a concomitant increase in extracellular α-synuclein of culture medium compared with those of the α-synuclein treatment group (Figure 2C). To exclude lysosomal degradation of α-synuclein by SH-SY5Y and internalization of α-synuclein by MSCs, a lysosomal inhibitor (bafilomycin) and endocytosis blocker (dynasore) were added to the SH-SY5Y and MSC co-cultures, respectively (Figure 1C,D). These drugs did not influence α-synuclein internalization or change the intracellular and extracellular α-synuclein levels (Figure 2C,D), indicating that soluble factors secreted from MSCs might be responsible for modulation of α-synuclein internalization. Incubation of α-synuclein fibrils for 24 hr and 48 hr decreased SH-SY5Y cell viability, whereas co-culture with MSCs recovered α-synuclein-induced cell viability (Figure 2E).

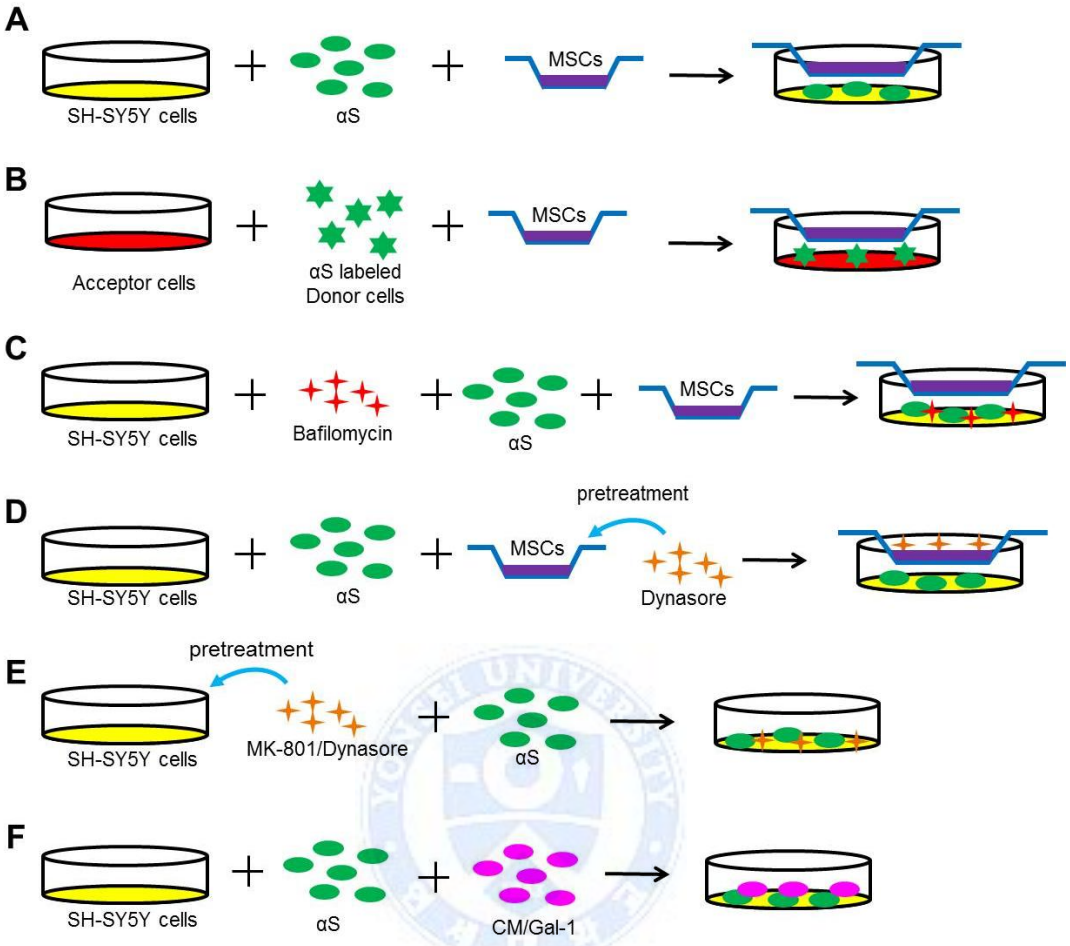


Figure 1. Schematic illustrations of in vitro experiments of α-synuclein transmission. (A) SH-SY5Y cells maintained on the bottom of a plate were co-cultured with MSCs using Costar transwell insert and simultaneously were treated with α-synuclein fibrils labeled with Alexa 488. (B) A donor-acceptor co-culture method. The donor cells labeled with α-synuclein were co-cultured on the top of the acceptor cells transfected with mCherry-tagged (Red). Simultaneously, these cells were co-cultured with MSCs using Costar transwell insert. (C,D) To exclude lysosomal degradation of α-synuclein by SH-SY5Y and internalization of α-synuclein by MSCs, bafilomycin was simultaneously added to the SH-SY5Y and MSC co-cultures (C) and MSCs were pre-treated with dynasore and then co-cultured with SH-SY5Y cells (D). (E) The effects of treatment with MK-801 or dynasore on internalization of α-synuclein. (F) The effects of MSC-CM, Gal-1 siRNA-treated MSC-CM, or Gal-1 treatment on internalization of α-synuclein.

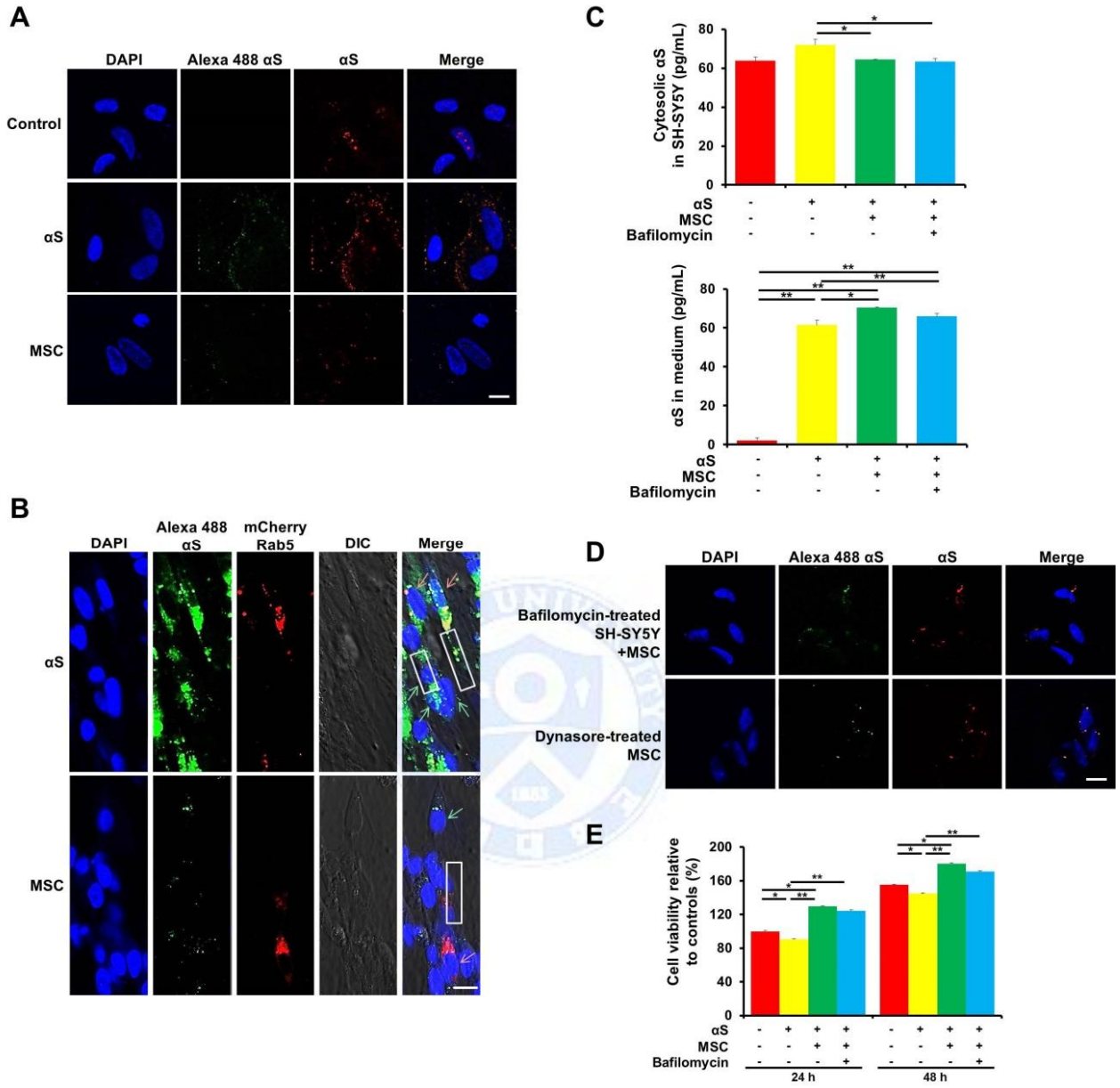


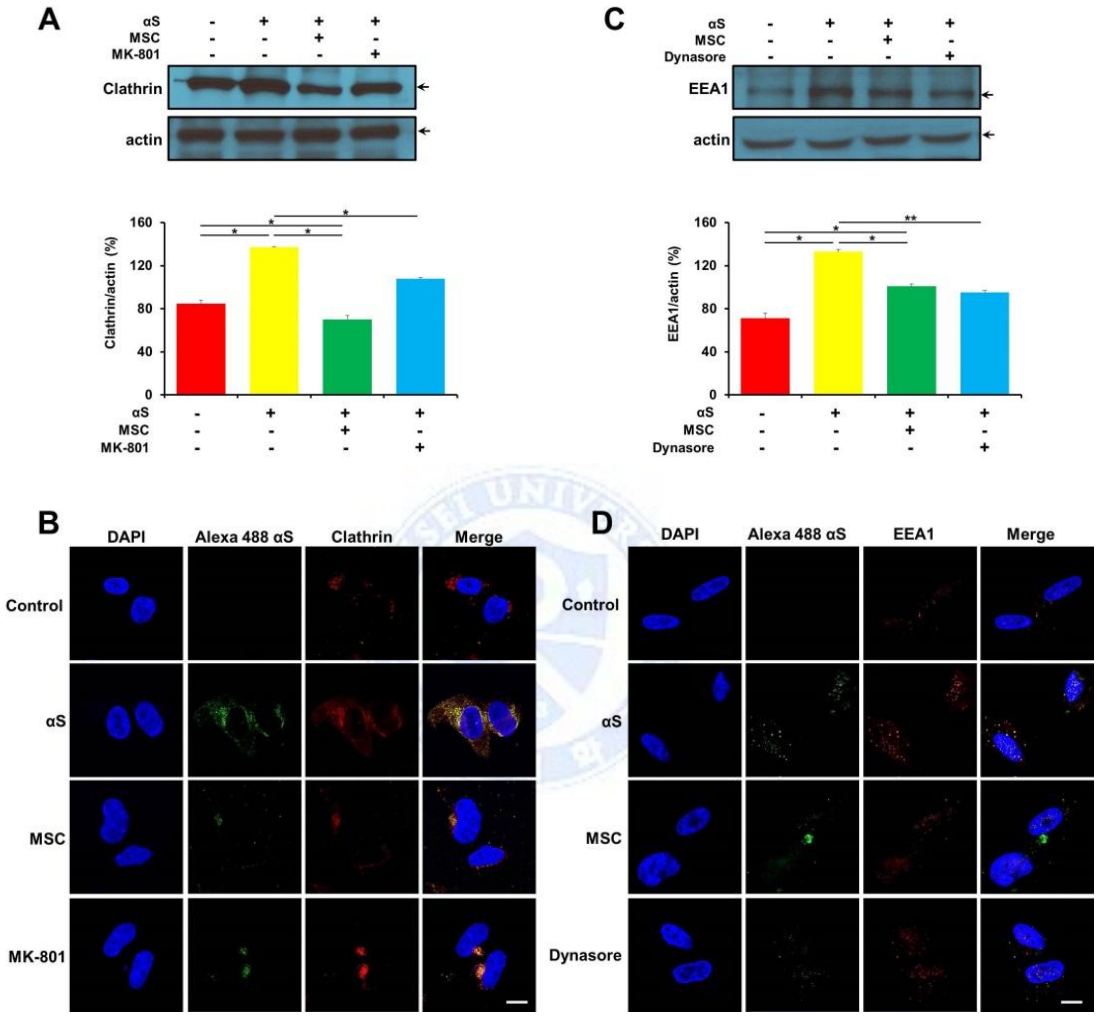
Figure 2. MSCs inhibit internalization and cell-to-cell transmission of extracellular α-synuclein. (A) Immunostaining for internalization of labeled α-synuclein (αS, green) in neuronal cells after treatment with α-synuclein or co-culture with MSCs (MSC). Scale bar, 10 μm. (B) A donor-acceptor co-culture method for transmission (white boxed area) of α-synuclein from donor cells (green arrows) to connected acceptor cells (red arrows) after either α-synuclein treatment alone (upper panel) or co-culture with MSCs (lower panel). Scale bar, 10 μm. (C) Quantification of internalized cytosolic α-synuclein and extracellular α-synuclein in the culture medium of the α-synuclein treatment group, the

co-culture group with MSCs, and the bafilomycin-treated MSC group (n=3, each group). (D) Immunostaining for internalization of labeled α-synuclein in neuronal cells co-cultured with MSCs after treatment with either bafilomycin or dynasore. Scale bar, 10 μm. (E) Quantification of cell viability in the α-synuclein group, the co-culture group with MSCs, and the bafilomycin-treated MSC group (n=3, each group). All data are presented as the means ± s.e (independent sample *t* test). $*P < 0.05$, $**P < 0.01$.

2. MSCs inhibit CME of extracellular α-synuclein fibrils through modulation of surface NMDA receptors

We examned the expression and immnucoreactivity of clathrin to determine the role of CME in α-synuclein internalization. When α-synuclein fibrils were incubated in SH-SY5Y cells, the expression of clathrin was significantly increased compared with that of the control. However, co-culutre with MSCs or treatment with MK-801 (Figure 1A,E), a noncompetitive NMDA receptor antagonist, significantly attenuated the expression of α-synuclein-induced clathrin (Figure 3A). Using confocal microscopy, we confirmed that co-culture with MSCs or treatment with MK-801 in α-synuclein-treated neuronal cells markedly decreased the immnuoreactivity of clathrin that was co-localized with α-synuclein (Figure 3B). Additionally, the expression of early endosome antigen 1 (EEA1) was increased significantly in α-synuclein-treated neuronal cells compared with that of the control. However, co-culure with MSCs or treatment with dysnasore (Figure 1A,E) significantly decreased the expression of EEA1, the level of which corresponded to that of an endocytosis inhibitor treatment (Figure 3C). Confocal microscopy showed that co-culture with MSCs or dynasore treatment in α-synuclein-treated neuronal cells markedly decreased the immnuoreactivity of EEA1 that was co-localized with α-synuclein (Figure 3D). Next, we identified whether MSCs inhibit the interaction between α-synuclein and NMDA receptors using extraction of cell surface membrane. α-Synuclein treatment in SH-SY5Y cells led to a significant attenuation in the expression of surface NR1 and NR2A subunits relative to control. However, co-culture with MSCs or MK-801treatment (Figure 1A,E) significantly increased the expression of α-synuclein-induced NR1 and NR2A subunits (Figure 3E,G). Moreover, immunocytochemical analysis showed that α-Synuclein treatment increased

immunoreactivity of surface NR1 and NR2A subunits that were co-localized with α-synuclein, whereas MSCs or MK801 treatment led to a decrease in immnuoreactivity of co-merged α-synuclein and surface NR1 and NR2A subunits (Figure 3F,H).



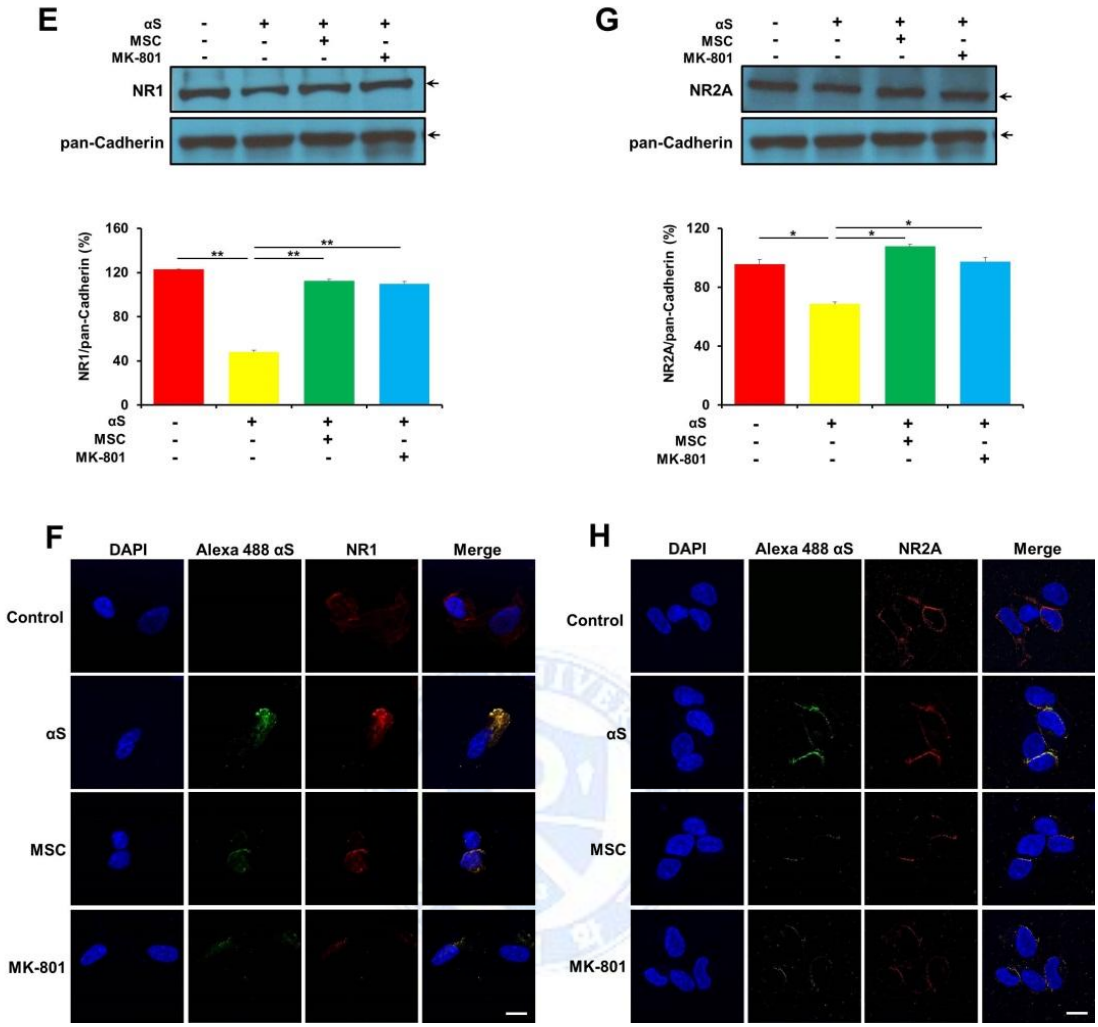


Figure 3. MSCs inhibit CME of extracellular α-synuclein fibrils via modulation of surface NMDA receptors. (A,B) Western blot for clathrin (A) and immunostaining of clathrin that is co-localized with α-synuclein (B) after either α-synuclein treatment alone, co-culture with MSCs (MSC), or MK-801 treatment (n=3, each group). Scale bar, 10 μm. (C,D) Western blot for EEA1 (C) and immunostaining of EEA1that is co-localized with α-synuclein (D) after either α-synuclein treatment alone, co-culture with MSCs, or dynasore treatment (n=3, each group). Scale bar, 10 μm. (E,F) Western blot for NR1 (E) and immunostaining of NR1 that is co-localized with α-synuclein (F) after either α-synuclein treatment alone, co-culture with MSCs, or MK-801 treatment (n=3, each group). Scale bar, 10 μm. (G,H) Western blot for NR2A (G) and immunostaining of NR2A that is co-localized with α-synuclein (H) after either α-synuclein treatment alone, co-culture with MSCs, or MK-801 treatment (n=3, each group). Arrowheads denote co-merged α-synuclein and these proteins.

Scale bar, 10 μm. All data are presented as the means ± s.e (independent sample *t* test). **P* < 0.05, ***P* < 0.01.

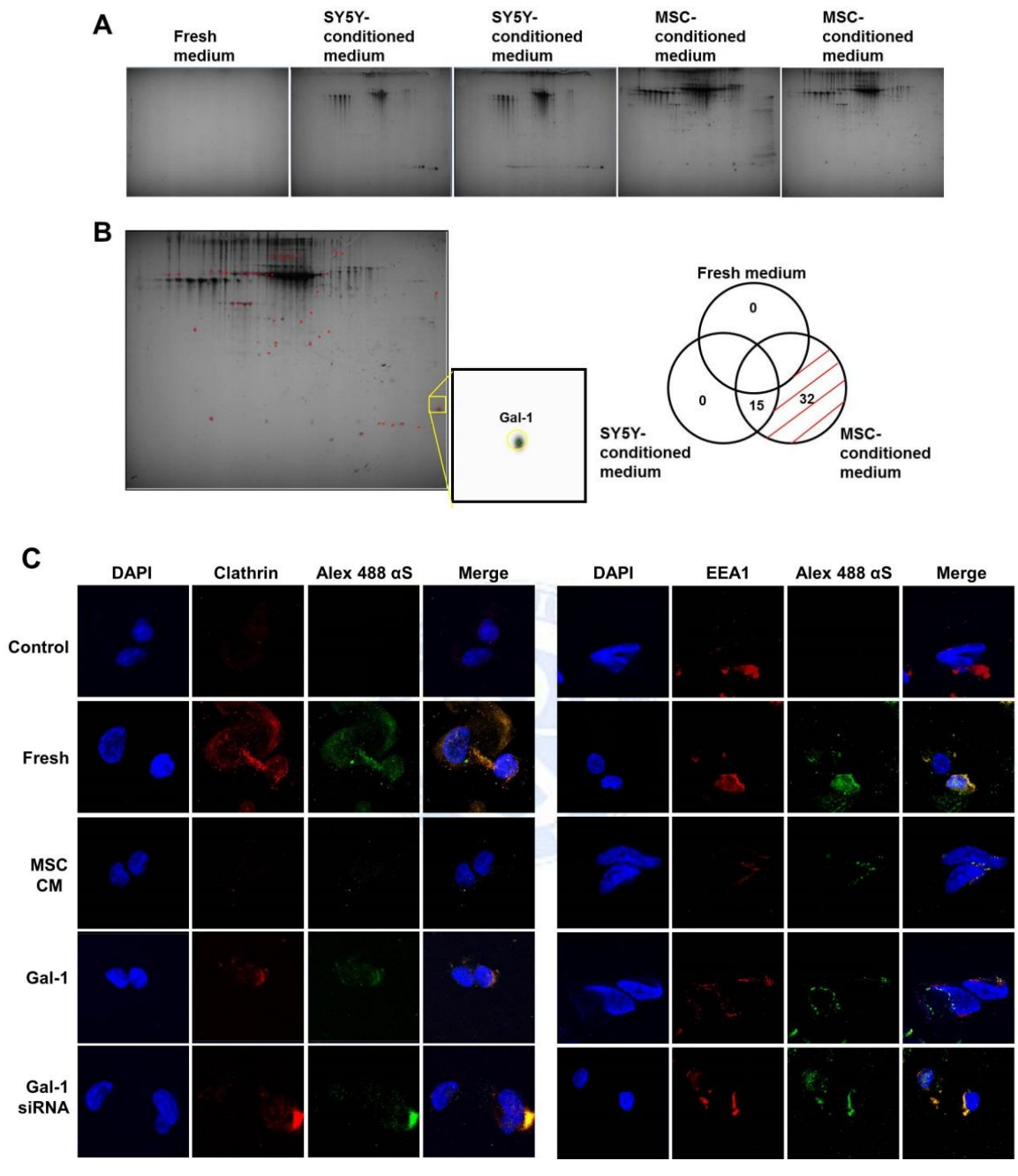
3. Identification and characterization of MSC-derived factors

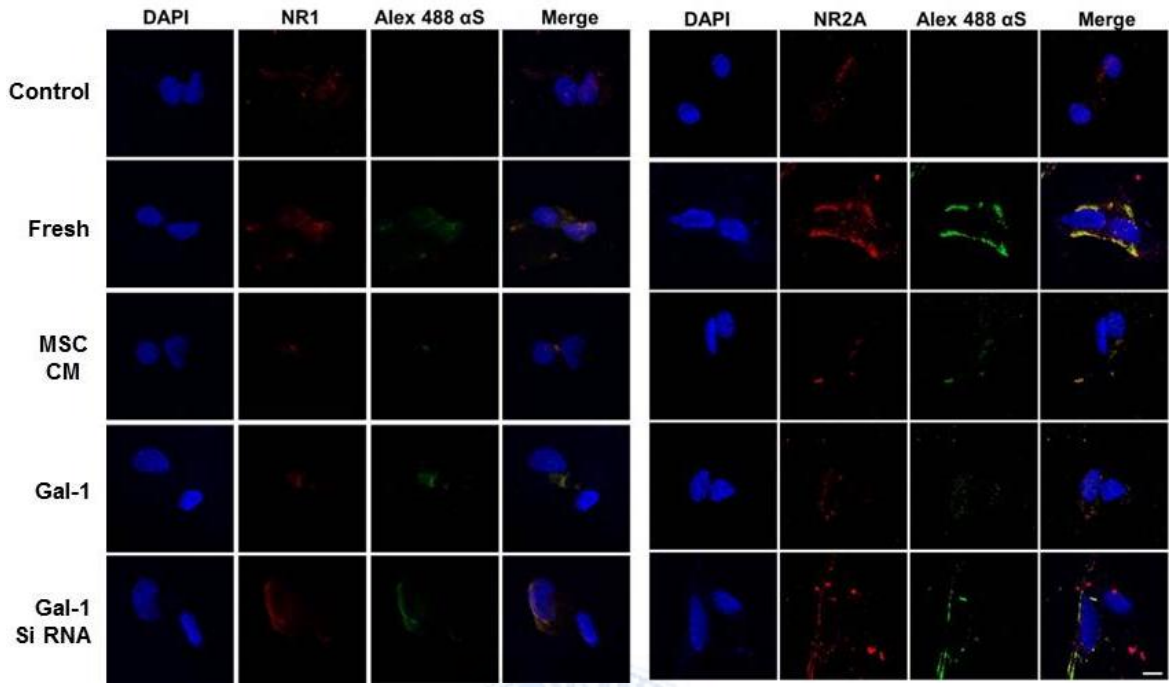
To determine proteins secreted from MSCs, we collected three independent samples of fresh medium, SH-SY5Y-conditioned media (CM), and MSC-CM for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and matrix-assisted laser desorption/ionization-time of flight mass spectrometer/mass spectrometer (MALDI-TOF/MS) proteomics (Figure 4A,B). Of the proteins expressed exclusively in MSC-CM (Table 1), we selected Gal-1 as candidates for α-synuclein modulation. We identified that Gal-1 was expressed within MSCs injected intravenously in α-synuclein-inoculated animals by showing that human specific nuclear mitotic apparatus protein (NuMA)-positive cells were co-immunostained with Gal-1 (Figure 5A) and that the expression of these proteins was significantly increased in MSCs-treated animals (Figure 5B). Next, we evaluated whether Gal-1 can modulate cell-to-cell transmission of α-synuclein (Figure 1F). Gal-1 treatment in α-synuclein-treated neuronal cells led to decreased internalization of labeled α-synuclein (Figure 4C) with a concomitant decrease in expressions of clathrin and EEA1 as well as increased expression of NR1 and NR2A (Figure 4D), which was followed by increased neuronal viability (Figure 4E). When MSCs were treated with Gal-1 siRNA (Figure 6), Gal-1 siRNA counteracted the inhibitory effect of MSCs on CME of α-synuclein via NMDA receptors (Figure 4C,D). Moreover, SH-SY5Y cell viability was prominently attenuated in the presence of Gal-1 siRNA-treated MSC-CM (Figure 4E). However, the intensity of α-synuclein aggregates, cell-to-cell transmission, and neuronal viability in Gal-1 siRNA-treated MSC-CM were not comparable to the corresponding values in fresh medium or SH-SY5Y-CM (Figure 4C–E), suggesting that other soluble factors may be involved in extracellular α-synuclein modulation.

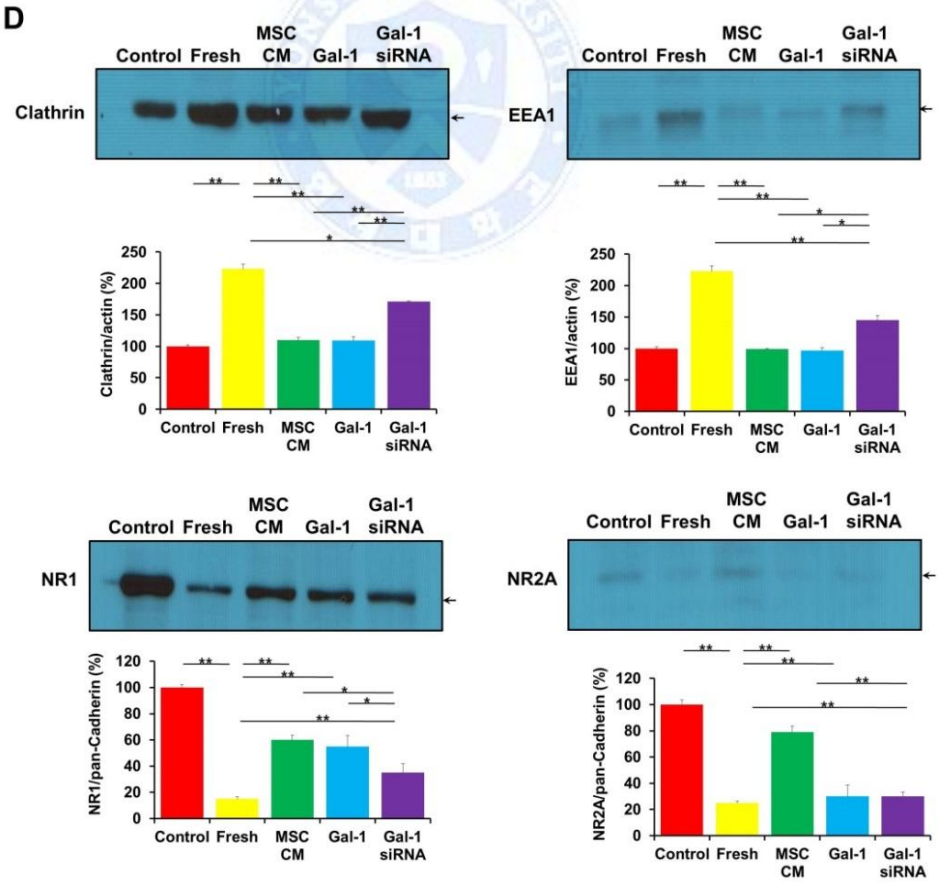
Table 1. Identification of differentially expressed proteins in MSC-CM.

No.	Accession No.	Protein name	pI	MW	Coverage (%)	Score
1	gi|4254297	Chain B, X-Ray Crystal Structure Of	4.8	21.2	75	139
2	gi|1578314	Structural And Electrophysiological	4.7	43.2	76	282
3	gi|1527750	ACTB protein	5.1	55.2	70	309
4	gi|5822007	Chain A, Gelatinase A	85.6	5	23	107
5	gi|178045	gamma-actin, partial	38.2	5.4	58	165
6	gi|2136109	ubiquitin carboxyl-terminal hydrolase	34.6	5.4	47	136
7	gi|4033042	septin-5 isoform 1	37.7	6	27	74
8	gi|30102	type I collagen	40.6	6.2	37	168
9	gi|4507877	vinculin isoform VCL	128.4	6.6	23	172
10	gi|7669550	vinculin isoform meta-VCL	128.8	6.7	20	135
11	gi|1869729	The Human Non-Classical Major	18.9	7.2	79	194
12	gi|184086	histone H2B.1, partial	22.7	9.5	64	122

pI: Isoelectric Point, MW: Molecular Weight


A
Fresh medium
SY5Y-conditioned medium
SY5Y-conditioned medium
MSC-conditioned medium
MSC-conditioned medium
B
Gal-1
Fresh medium
0
0
15
32
SY5Y-conditioned medium
MSC-conditioned medium
C
DAPI
Clathrin
Alex 488 αS
Merge
DAPI
EEA1
Alex 488 αS
Merge
Control
Fresh
MSC CM
Gal-1
Gal-1 siRNA


DAPI
NR1
Alex 488 αS
Merge
DAPI
NR2A
Alex 488 αS
Merge
Control
Fresh
MSC CM
Gal-1
Gal-1 Si RNA


D
Control
Fresh
MSC CM
Gal-1
Gal-1 siRNA
Clathrin
EEA1
Clathrin/actin (%)
EEA1/actin (%)
NR1
NR2A
NR1/pan-Cadherin (%)
NR2A/pan-Cadherin (%)

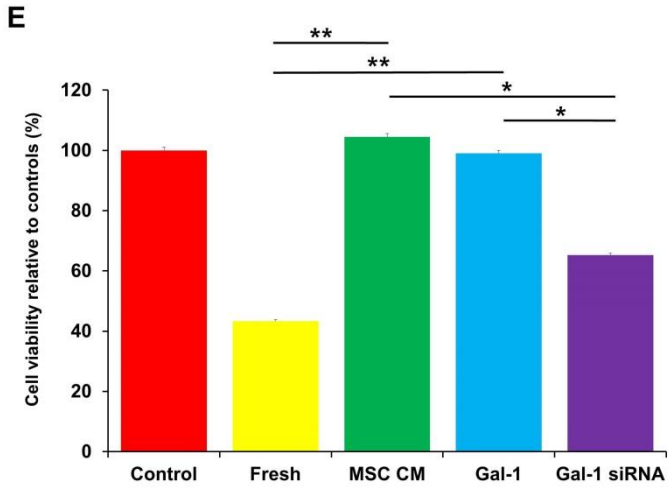


Figure 4. Gal-1 plays an important role in transmission control of extracellular α-synuclein. (A,B) 2D-PAGE and MALDI-TOF/MS proteomics approaches from MSC-CM**.** On the 2D-PAGE gel of total protein extracted from each medium at 72 h, spot intensities and patterns were largely similar between independent samples from the SH-SY5Y-CM group and MSC-CM group. (A) Fresh medium contained 6 discernable polypeptides, SH-SY5Y-CM contained 87 and 96 discernable polypeptides, and MSC-CM contained 189 and 157 discernable polypeptides by silver staining. (B) The analysis identified 47 spots in MSC-CM that differed significantly by at least twofold in expression level compared with fresh medium or SH-SY5Y-CM. The area shown in the yellow box in the left panel is enlarged in the right panels. Gal-1 protein was one of them expressed by yellow circle in enlarged image. A Venn diagram showed that MSC-CM contained 32 spots, including Gal-1, which were not shared by fresh medium and SH-SY5Y-CM. (C) Immunostaining for clathrin, EEA1, and surface NR1 and NR2A in neuronal cells that are co-localized with α-synuclein (αS) in the presence of fresh medium, MSC-CM, Gal-1 treatment, or Gal-1 siRNA treated MSC-CM. Scale bar, 10 μm. (D) Western blot for clathrin, EEA1, and surface NR1 and NR2A in neuronal cells after treatment with either Gal-1 or Gla-1 siRNA treated MSC-CM compared with fresh medium or MSC-CM (n=3, each group). (E) A viability assay in neuronal cells after αS fibrils were treated with either Gal-1 or Gal-1 siRNA treated MSC-CM compared with fresh medium or MSC-CM (n=5, each group). All data are presented as the menas ± s.e. $*P < 0.05$, $**P < 0.01$.

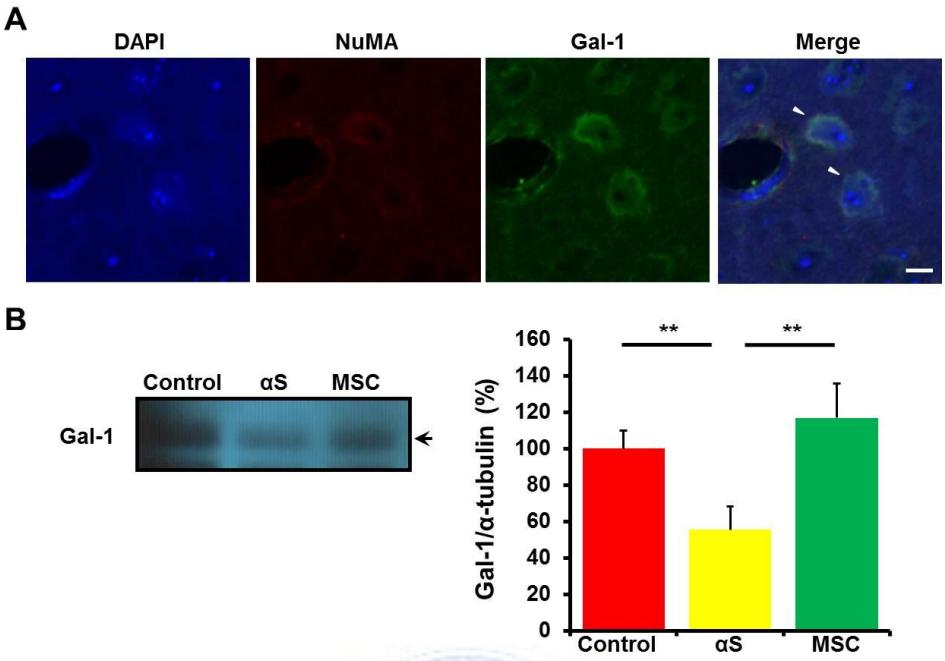


Figure 5. Gal-1 expresses within MSCs injected intravenously in α-synuclein-inoculated animals. NuMA-positive cells were co-immunostained with in the cortex of MSCs-treated animals (A), and the expression of Gal-1 was significantly increased in MSCs-treated animals (B). Arrowheads denote co-localization of NuMA with Gal-1. Scale bar represents 20 μm.

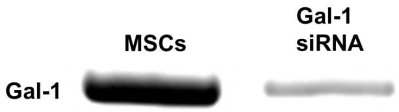


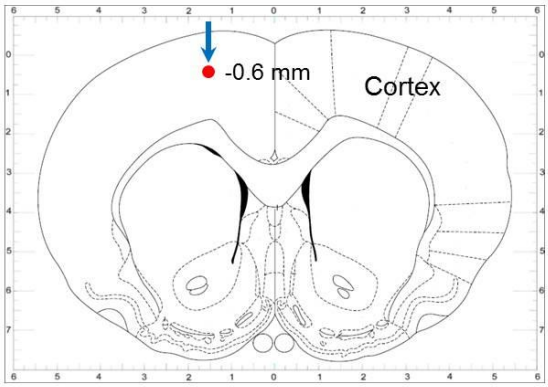
Figure 6. RT-PCR analysis of Gal-1 in MSCs. Transfection of MSCs with a Gal-1siRNA construct effectively downregulated endogenous expression of Gal-1 mRNA.

4. Short-term effects of MSCs on cell-to-cell transmission of extracellular α-synuclein in α-synuclein-inoculated animals

Following stereotaxic inoculation of Alexa Fluor 488-labeled α-synuclein fibrils into the cortex of mice (Figure 7A,B), we examined internalized α-synuclein that was detected as fluorescent punctate within the cytoplasm at 7 days after α-synuclein inoculation. The immunoreactivity of α-synuclein was more densely observed in the cortical areas neighboring the inoculation site and extensively

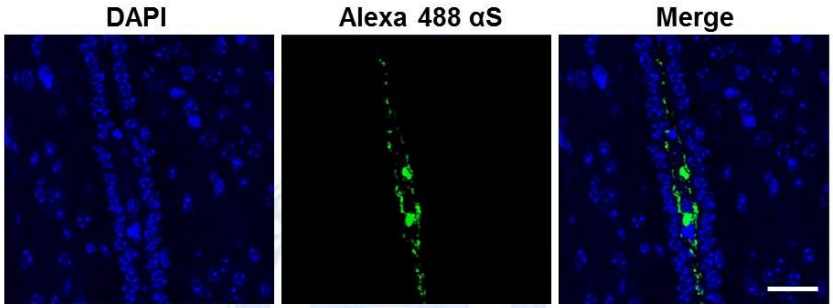
detected from the ipsilateral cortex to the contralateral hemisphere (Figure 8A). However, MSC treatment markedly decreased the density of internalized α-synuclein in both ipsilateral and contralateral hemispheres (Figure 8A). Moreover, we examined the immunoreactivity of phosphorylated α-synuclein to evaluate whether exogenous α-synuclein induces pathogenic α-synuclein. The phosphorylated α-synuclein was exclusively immunostained in ipsilateral and contralateral hemispheres of α-synuclein-inoculated animals, and this immunoreactivity was observed in neurons regardless of exogenous α-synuclein (Figure 8B). However phosphorylated α-synuclein immunoreactivity was not observed in MSC-treated animals (Figure 8B). The ELISA analysis showed that MSC treatment significantly attenuated the expression of cytosolic α-synuclein in both ipsilateral and contralateral hemispheres of α-synuclein inoculation compared with that of α-synuclein-treated mice (Figure 8C). In addition, MSC treatment in α-synuclein-inoculated brain significantly attenuated phosphorylated α-synuclein expression (Figure 8D). Next, we examined whether MSC administration modulates CME of extracellular α-synuclein fibrils through modulation of surface NMDA receptors. An inoculation of α-synuclein fibrils led to increased expression of clathrin in both ipsilateral and contralateral hemispheres of inoculation, whereas MSC treatment significantly attenuated expression of this protein (Figure 8E). Immunohistochemical analysis showed that MSC treatment led to a decrease in the immunoreactivity of labeled α-synuclein and clathrin in both ipsilateral and contralateral hemispheres (Figure 9). As a result of CME inhibition, MSC administration in α-synuclein-inoculated mice significantly decreased expression of EEA1 (Figure 8E) and the immunoreactivity of labeled α-synuclein and EEA1 in both ipsilateral and contralateral hemispheres (Figure 9). Additionally, MSC administration led to a significant increase in the expression of α-synuclein-induced surface NR1 and NR2A subunits (Figure 8E) and a decrease in immunoreactivity of surface NR1 and NR2A subunits that were co-localized with α-synuclein (Figure 9) in both ipsilateral and contralateral hemispheres of α-synuclein-inoculated mice. Consequently, α-synuclein inoculation led to markedly increased expression of cleaved caspase-3 and the immunoreactivity of terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling (TUNEL) in the ipsilateral hemisphere relative to controls; however, MSC or Gal-1 treatment notably decreased the expression of cleaved caspase-3 and the number of TUNEL-positive neurons (Figure 8F,G).

A

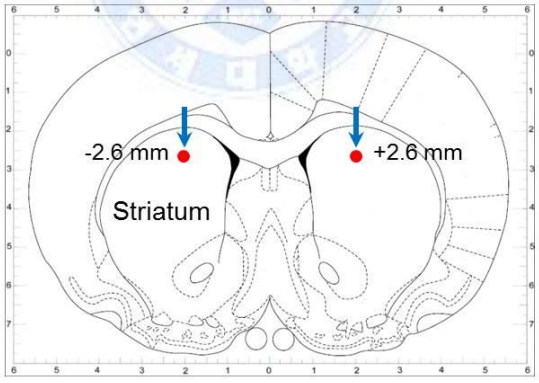


+0.4 mm posterior to the bregma

B



C



+0.2 mm posterior to the bregma

D

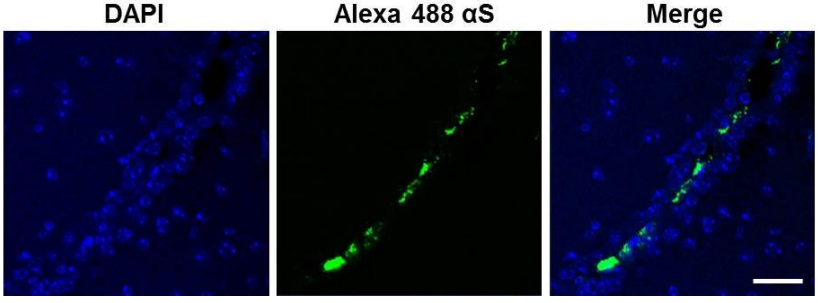
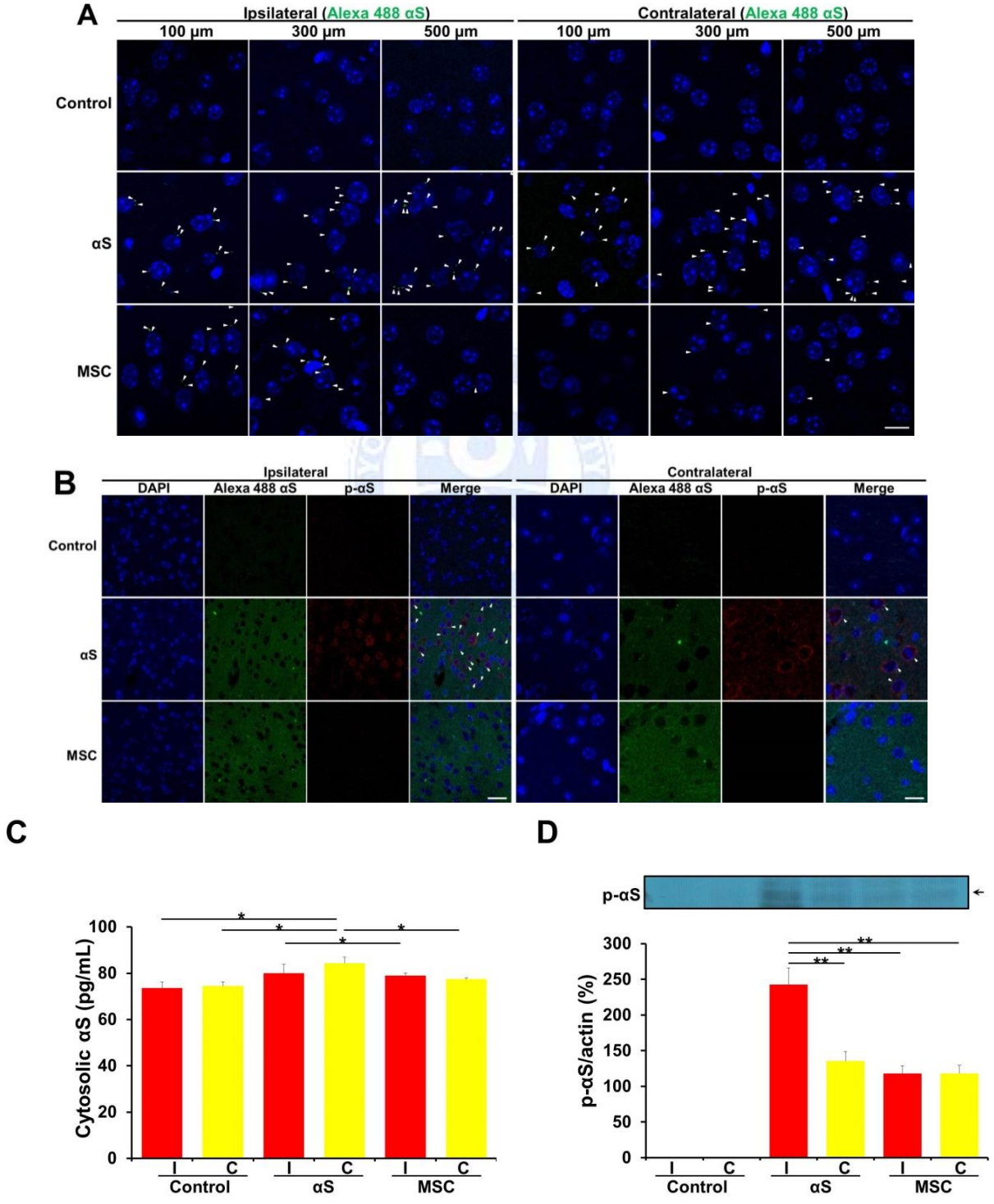
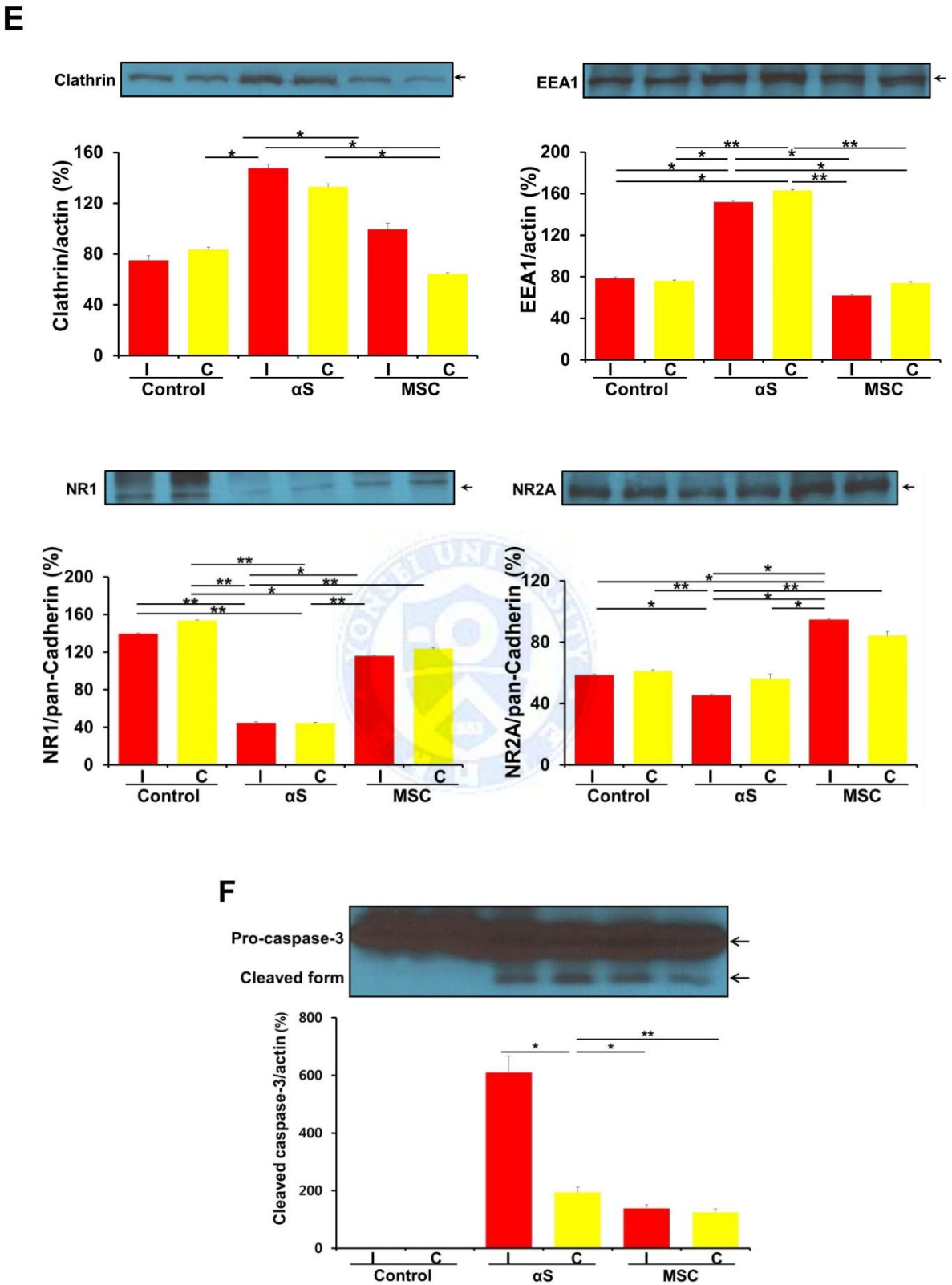


Figure 7. Schematic illustration of stereotaxic injections. Alexa Fluor 488-labeled α-synuclein fibrils (red circle) were inoculated stereotaxically into the right cortex (A) and the dorsal striatum (C). Following stereotaxic inoculation of α-synuclein, labeled α-synuclein fibrils were noted in the cortex (B) and striatum (D). Scale bar represents 10 μm.




E
Clathrin
EEA1
Clathrin/actin (%)
EEA1/actin (%)
NR1
NR2A
NR1/pan-Cadherin (%)
NR2A/pan-Cadherin (%)
I
C
Control
αS
MSC
F
Pro-caspase-3
Cleaved form
Cleaved caspase-3/actin (%)

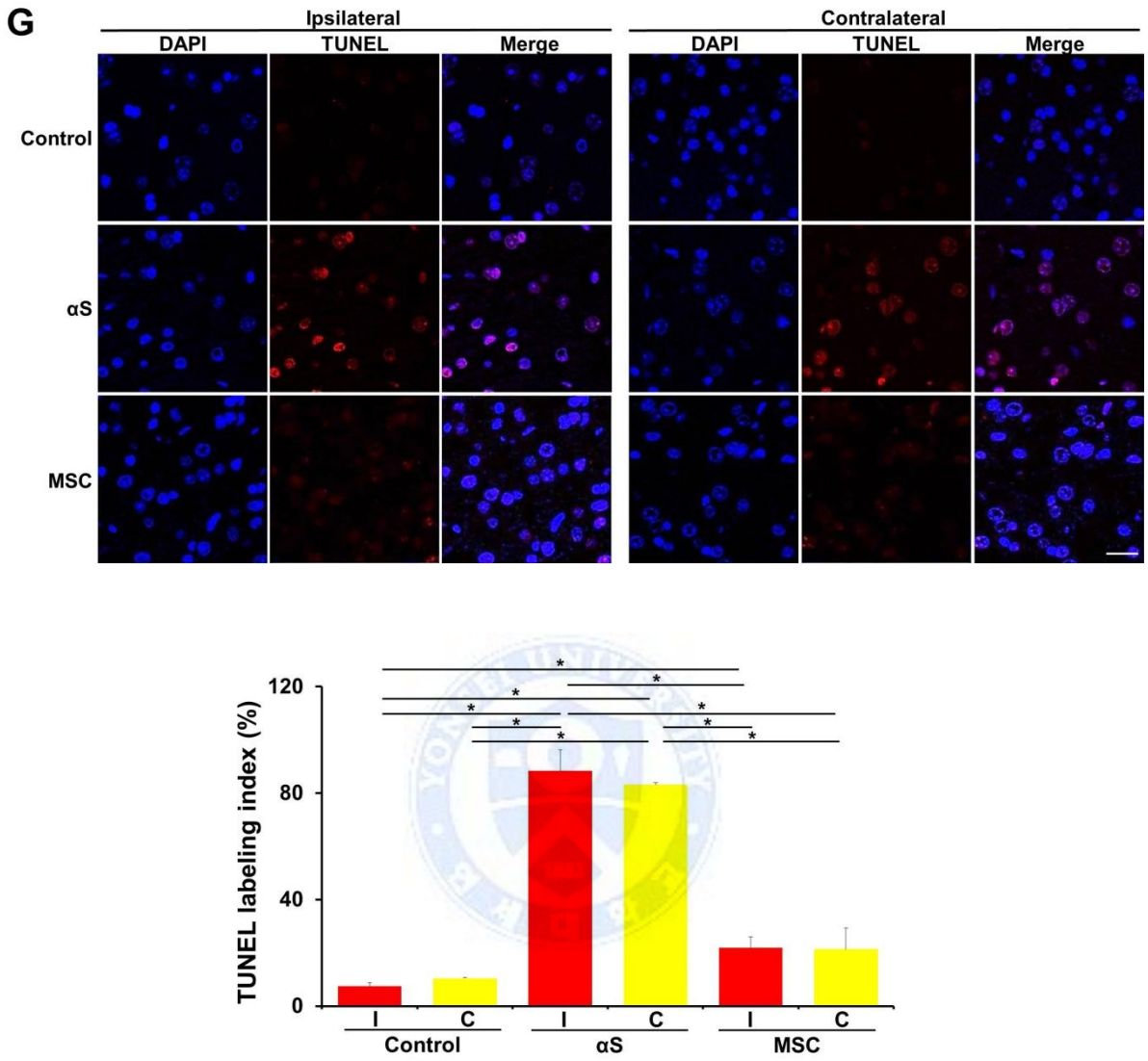
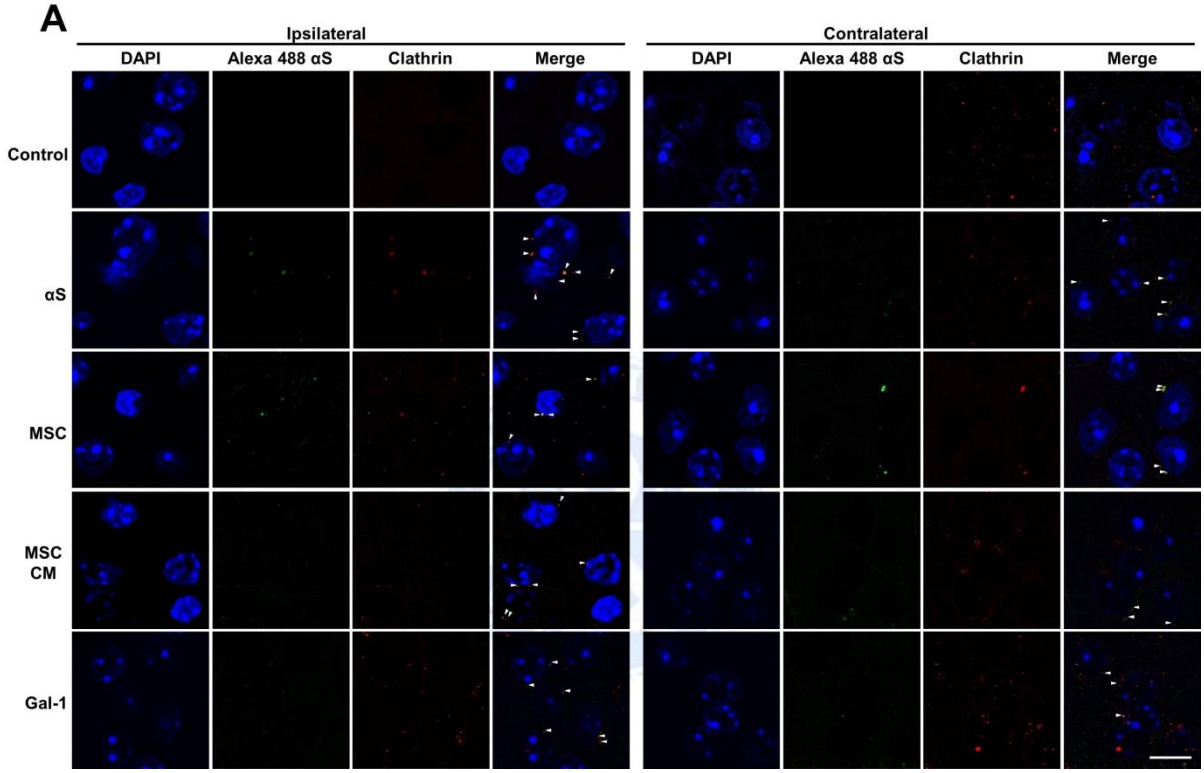
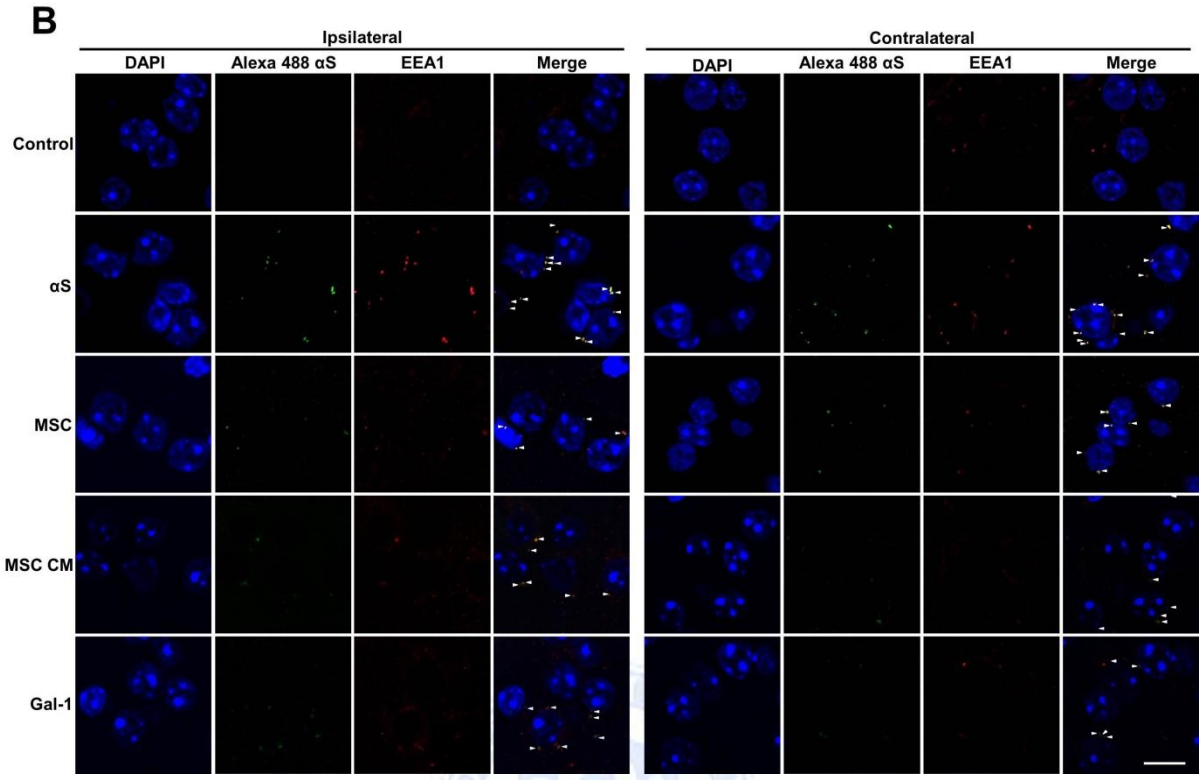
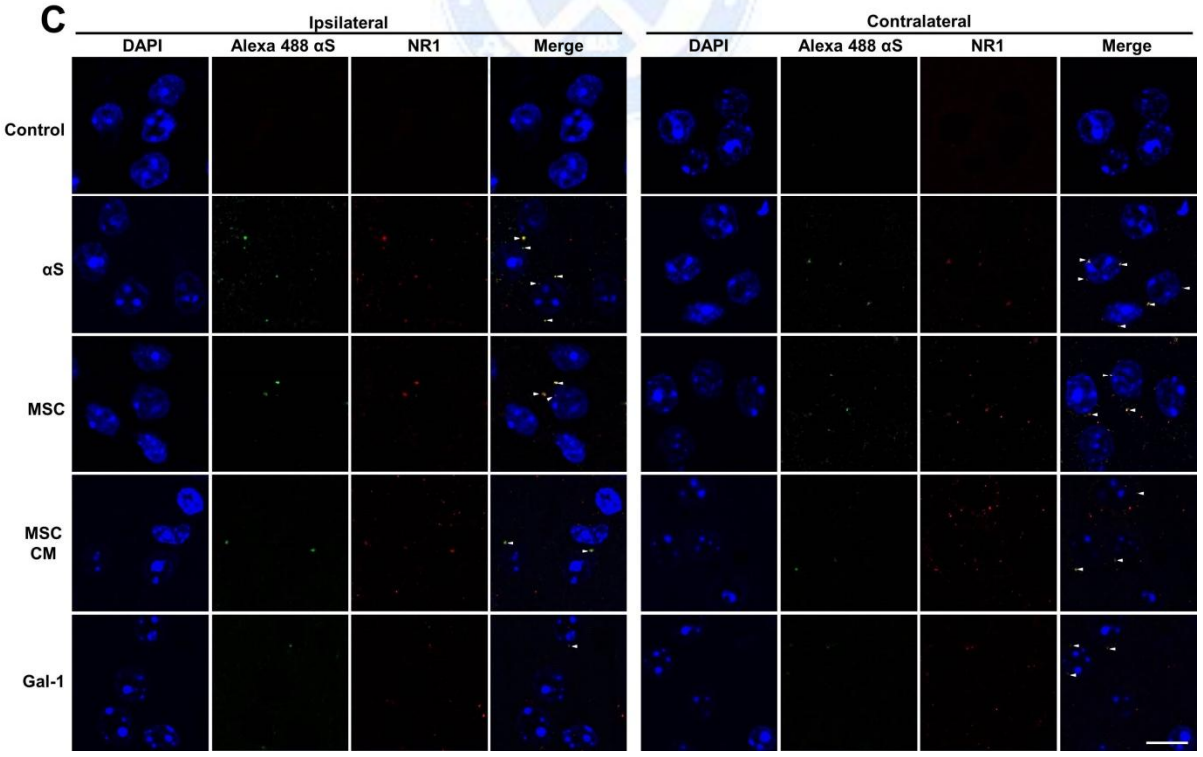


Figure 8. Short-term effects of MSCs on extracellular α-synuclein transmission. (A) Immunostaining for internalized Alexa 488 labeled α-synuclein (αS), showing that the density and extent of propagated αS in ipsilateral and contralateral hemispheres were less prominent in mice receiving MSCs (MSC) compared with αS alone. Scale bar, 10 μm. Arrowheads denote labeled αS in the cortical areas. (B) The immunoreactivity of phosphorylated α-synuclein (p-αS) was exclusively noted in αS-inoculated animals, whereas this immunoreactivity was not observed in MSC-treated animals. Scale bar, 10 μm. Arrowheads denote co-localization of Alexa 488 labeled αS with p-αS. (C) Quantification of cytosolic αS in ipsilateral and contralateral hemispheres of mice receiving MSCs compared with αS alone (n = 5, per group). (D) Western blot for p-αS in ipsilateral and contralateral hemispheres of mice receiving MSCs compared with αS alone (n = 5, per group). (E) Western blot for

clathrin, EEA1, NR1 subunit, and NR2A subunit in ipsilateral and contralateral hemispheres of mice receiving MSCs compared with αS alone (n = 5, per group). (F,G) Western blot for pro- and cleaved caspase-3 (F) and immunostaining of TUNEL (G) in mice after treatment with MSCs compared with αS alone. Scale bar, 10 μm. All data are presented as the menas ± s.e. *$P < 0.05$, **$P < 0.01$. I: ipsilateral, C: contralateral.




B
Ipsilateral
Contralateral
DAPI
Alexa 488 αS
EEA1
Merge
Control
αS
MSC
MSC CM
Gal-1


C
Ipsilateral
Contralateral
DAPI
Alexa 488 αS
NR1
Merge
Control
αS
MSC
MSC CM
Gal-1

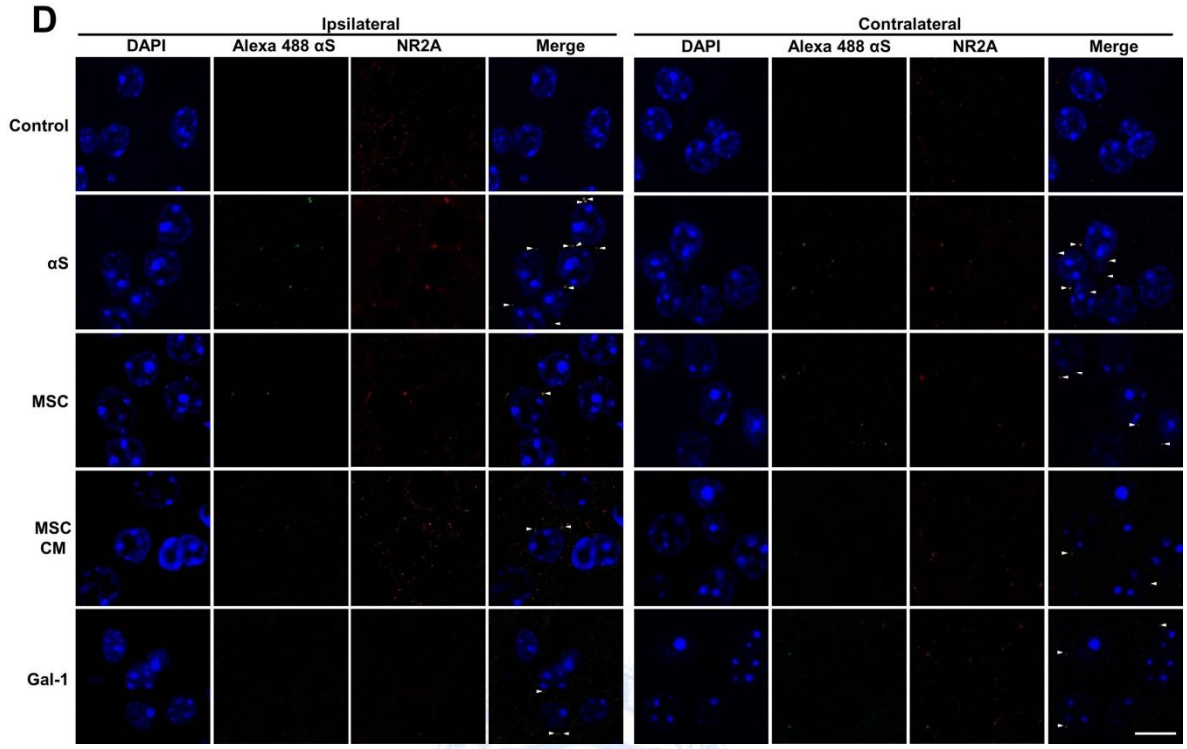
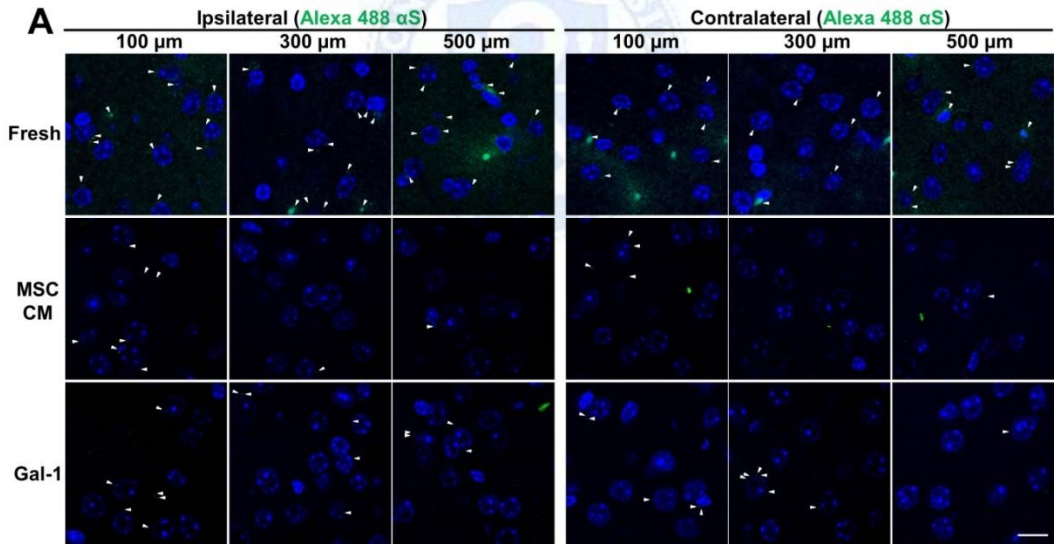


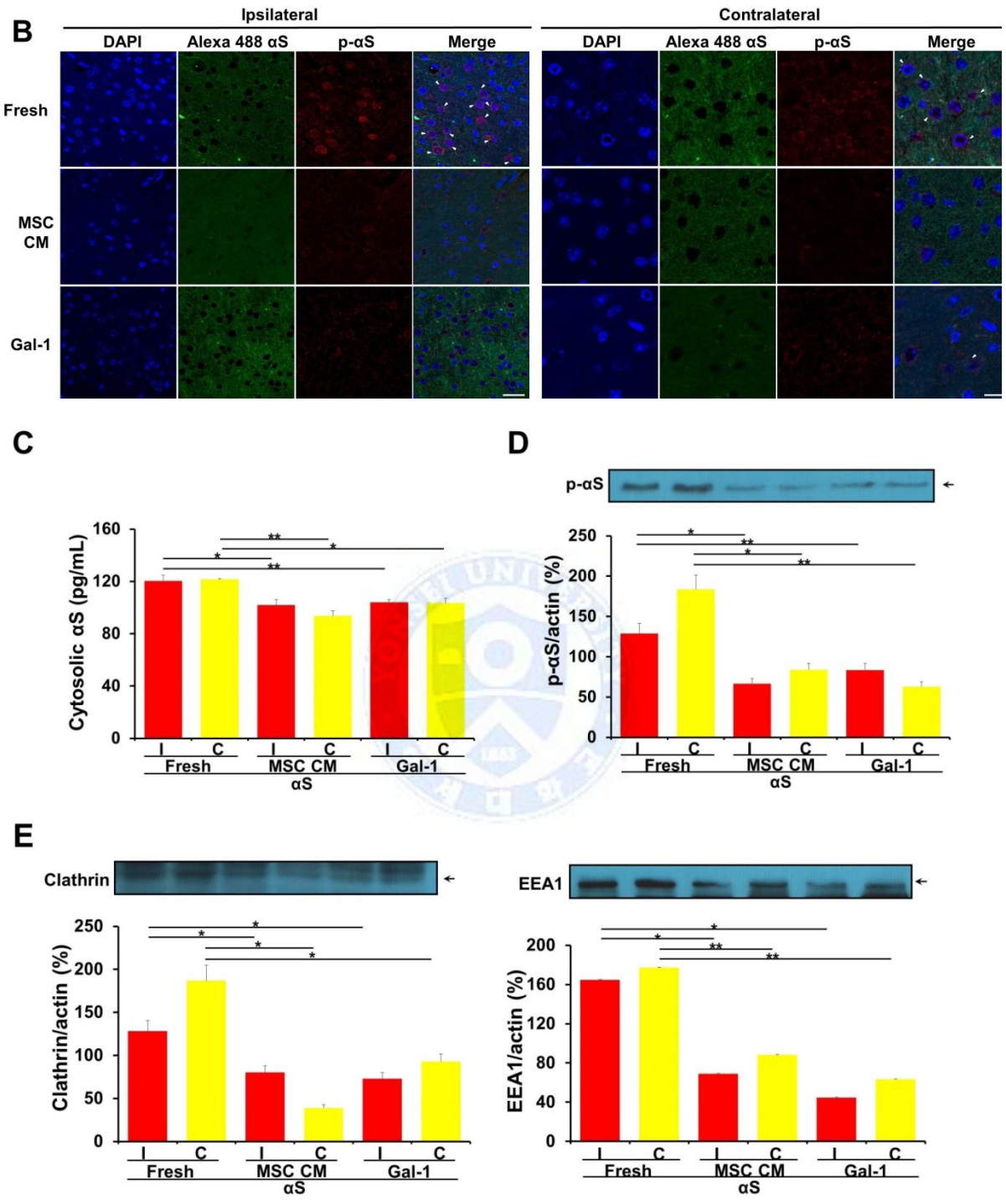
Figure 9. Treatment with either MSCs or Gal-1 modulates clathrin mediated endocytosis of extracellular α-synuclein fibrils through modulation of surface NMDA receptors. Immunostaining of clathrin (A), EEA1 (B), NR1 (C), and NR2A (D) that is co-localized with α-synuclein in ipsilateral and contralateral hemispheres of mice receiving MSCs (MSC), MSC-CM, or Gal-1 compared with α-synuclein alone. An inoculation of α-synuclein fibrils led to an increase in immunoreactivity of clathrin, EEA1, NR1, and NR2A that is co-localized with α-synuclein, whereas treatment with either MSCs or Gal-1 attenuated immunoreactivity of co-merged α-synuclein and these proteins (white arrowheads). Scale bar, 10 μm.

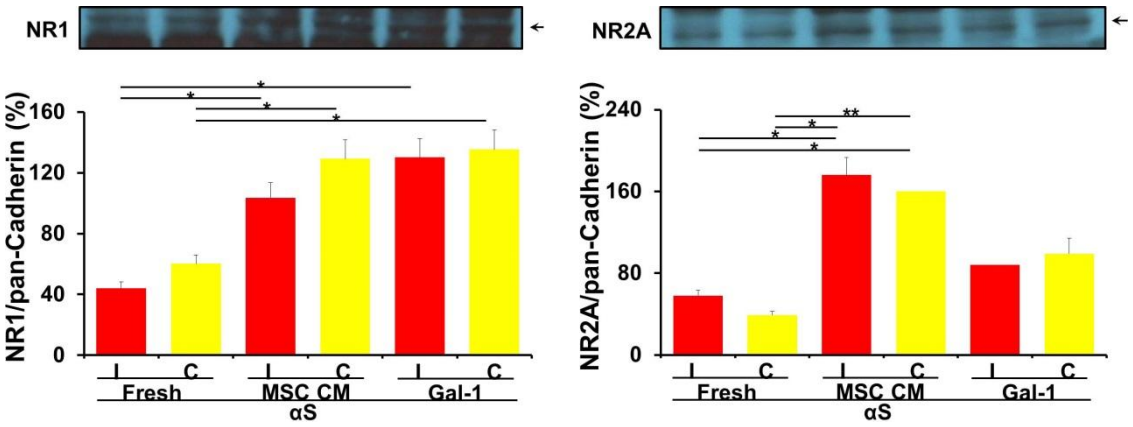
5. Cell-to-cell transmission of extracellular α-synuclein is decreased by Gal-1 in α-synuclein-inoculated animals

We evaluated whether Gal-1 can modulate α-synuclein transmission in in α-synuclein-inoculated animals. Inhibition of α-synuclein propagation was similarly observed following MSC-CM or Gal-1 treatment (Figure 10A). Additionally, MSC-CM or Gal-1 treatment markedly decreased the immunoreactivity of phosphorylated α-synuclein in ipsilateral and contralateral hemispheres of α-

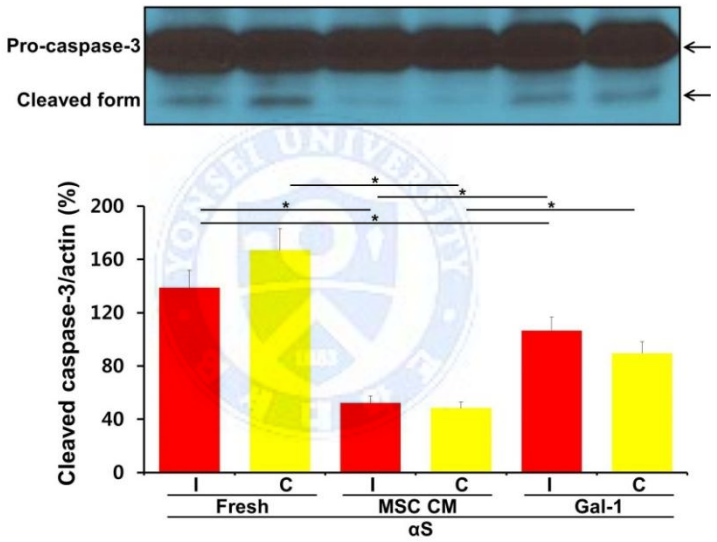
synuclein-inoculated animals (Figure 10B). The ELISA analysis confirmed that the expression of cytosolic and phosphorylated α-synuclein was significantly decreased in the ipsilateral and contralateral hemispheres following MSC-CM or Gal-1 (Figure 10C,D). MSC-CM or Gal-1 treatment significantly attenuated clathrin expression (Figure 10E) and immunoreactivity (Figure 9) in both ipsilateral and contralateral hemispheres of inoculation, which was accompanied by decreased expression of EEA1 (Figure 10E) and the immunoreactivity of labeled α-synuclein and EEA1 (Figure 9). MSC-CM or Gal-1 treatment inhibited CME of extracellular α-synuclein fibrils through modulation of surface NMDA receptors; however, Gal-1 treatment did not show a significant interaction with surface NR2A subunit (Figure 10E). Consequently, MSC or Gal-1 treatment notably decreased the expression of cleaved caspase-3 and the number of TUNEL-positive neurons in α-synuclein-inoculated animals (Figure 10F-H).



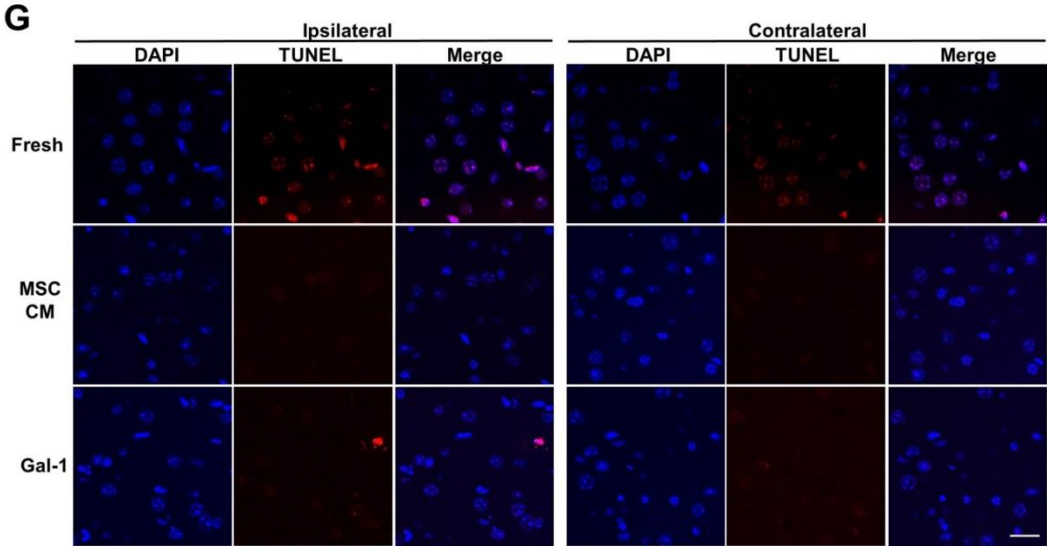

B
Ipsilateral
Contralateral
DAPI
Alexa 488 αS
p-αS
Merge
Fresh
MSC CM
Gal-1
C
Cytosolic αS (pg/mL)
D
p-αS
p-αS/actin (%)
I
C
Fresh
MSC CM
Gal-1
αS
E
Clathrin
Clathrin/actin (%)
EEA1
EEA1/actin (%)


NR1
NR2A
NR1/pan-Cadherin (%)
NR2A/pan-Cadherin (%)
0
40
80
120
160
240
I
C
Fresh
MSC CM
Gal-1
αS

F


Pro-caspase-3
Cleaved form
Cleaved caspase-3/actin (%)
0
40
80
120
160
200
I
C
Fresh
MSC CM
Gal-1
αS

G


Ipsilateral
Contralateral
DAPI
TUNEL
Merge
Fresh
MSC CM
Gal-1

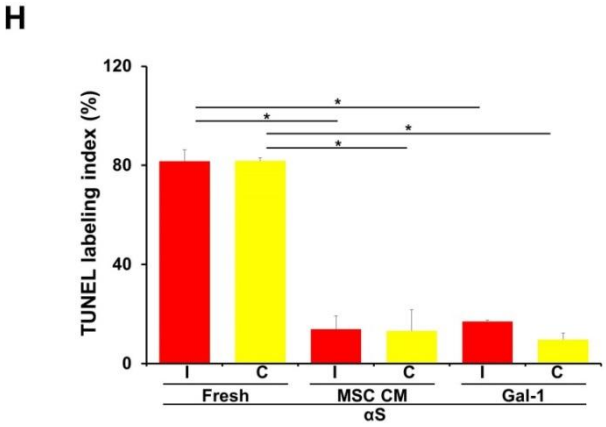
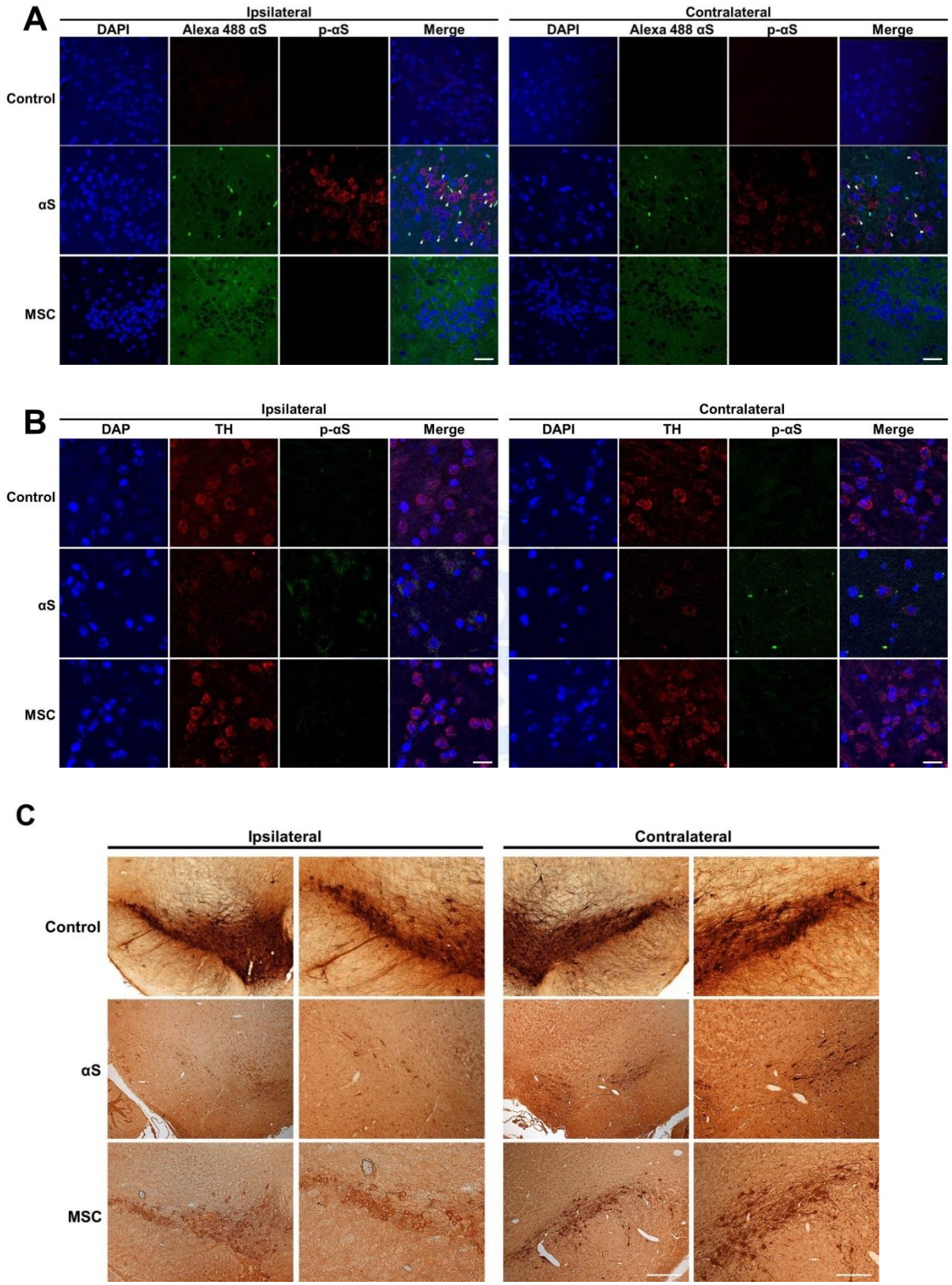
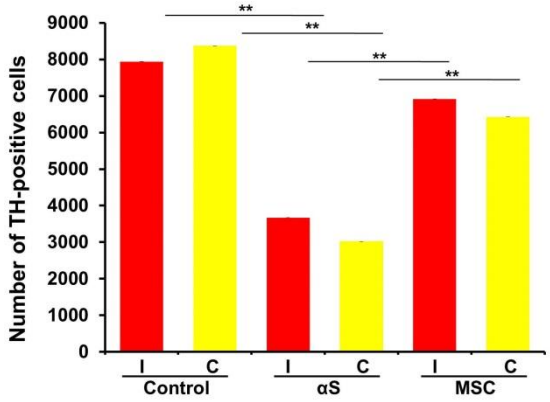


Figure 10. **Treatment with either MSC-CM or Gal-1 modulates clathrin mediated endocytosis of extracellular α-synuclein fibrils and reduces the cell-to-cell transmission.** (A) Immunostaining for internalized Alexa 488 labeled α-synuclein (αS), showing that the density and extent of propagated αS in ipsilateral and contralateral hemispheres were less prominent in mice receiving MSC-CM or Gal-1. Scale bar, 10 μm. Arrowheads denote Alexa 488 labeled αS in the cortical areas neighboring the inoculation site. (B) Immunostaining of Alexa 488 labeled αS and phosphorylated α-synuclein (p-αS) in ipsilateral and contralateral hemispheres were less prominent in treatment with either MSC-CM or Gal-1 compared with fresh medium. Scale bar, 10 μm. Arrowheads denote co-localization of Alexa 488 labeled αS with p-αS. (C) Quantification of cytosolic αS in ipsilateral and contralateral hemispheres of mice receiving MSC-CM or Gal-1 (n = 5, per group). (D) Western blot for p-αS in ipsilateral and contralateral hemispheres of mice receiving MSC-CM or Gal-1 (n = 5, per group). (E) Western blot for clathrin, EEA1, NR1 subunit, and NR2A subunit in ipsilateral and contralateral hemispheres of mice receiving MSC-CM or Gal-1 (n = 5, per group). (F,G) Western blot for pro- and cleaved caspase-3 (F), immunostaining of TUNEL (G), and TUNEL labelling index (H) in mice after treatment with MSC-CM or Gal-1. Scale bar, 10 μm. All data are presented as the menas ± s.e. *P < 0.05, **P < 0.01. I: ipsilateral, C: contralateral.

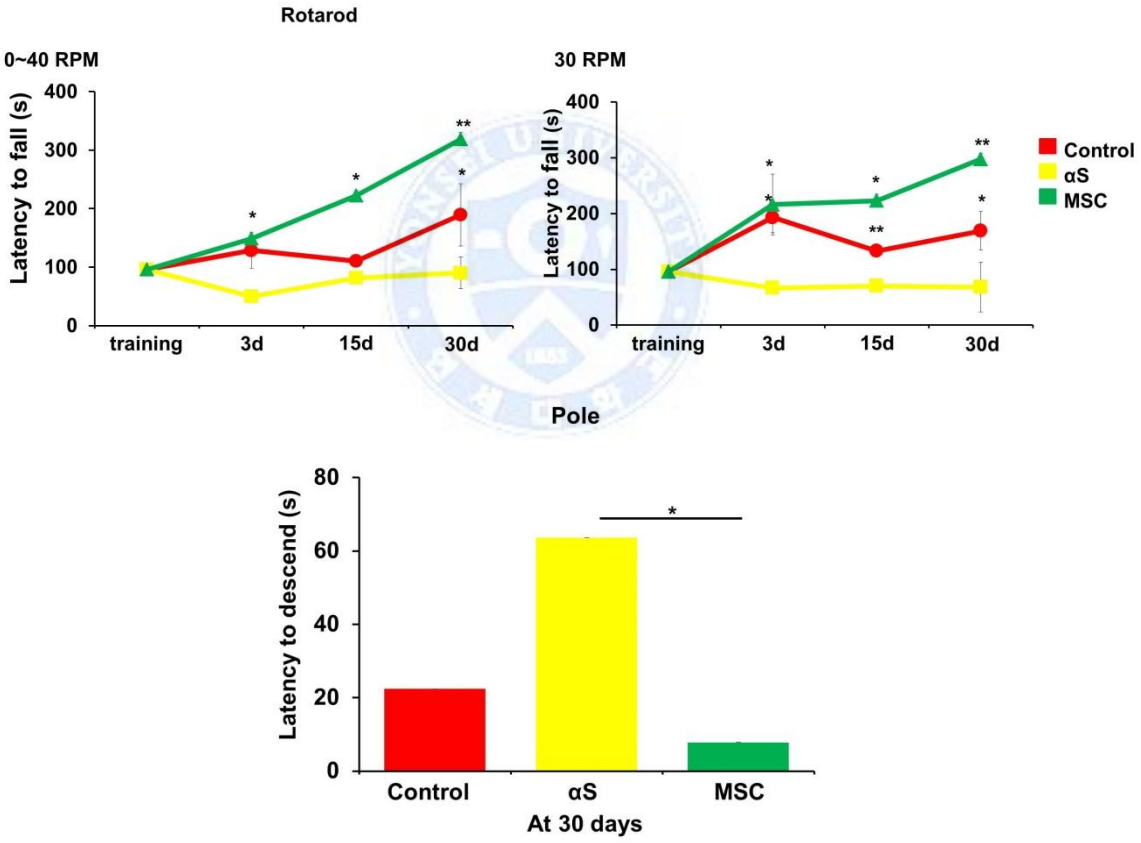
6. Long-term effects of MSCs on transmission of extracellular α-synuclein in α-synuclein-inoculated animals

Following stereotaxic inoculation of Alexa Fluor 488-labeled α-synuclein fibrils into the dorsal striatum of mice (Figure 7C,D), we assessed dopaminergic neuronal loss in the substantia nigra (SN) of the midbrain and behavioral deficits at 30 days after α-synuclein inoculation. α-Synuclein inoculation in the striatum markedly increased the immunoreactivity of phosphorylated form of α-synuclein in the midbrain (Figure 11A) as well as dopaminergic neurons (Figure 11B), which was accompanied by a significant decrease in the number of tyrosine hydroxylase (TH)-positive neurons in the SN (Figure 11C). On behavioral analysis, α-synuclein inoculation led to progressive patterns of the latency to fall on the Rotarod test and increased latency to descend at 30 days after α-synuclein inoculation on fall test compared to the control group (Figure 11D). However, MSC treatment in α-synuclein-inoculated animals significantly attenuated the immunoreactivity of phosphorylated form of α-synuclein in the midbrain (Figure 11A) as well as dopaminergic neurons (Figure 11B). Moreover, MSC treatment decreased significantly dopaminergic neuronal loss in the SN (Figure 11C) and led to restoration of impaired motor coordination and balance on the Rotarod test and pole test (Figure 11D). In addition, phosphorylated α-synuclein was observed in the striatal areas neighboring the inoculation site and extensively detected in the bilateral parietal and entorhinal cortices at 30 days after α-synuclein inoculation (Figure 11E). However, MSC treatment significantly attenuated the immunoreactivity of phosphorylated α-synuclein in these areas (Figure 11E).


A
Ipsilateral
Contralateral
DAPI
Alexa 488 αS
p-αS
Merge
Control
αS
MSC
B
Ipsilateral
Contralateral
DAP
TH
p-αS
Merge
Control
αS
MSC
C
Ipsilateral
Contralateral
Control
αS
MSC


Number of TH-positive cells
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
**
**
**
**
I
C
I
C
I
C
Control
αS
MSC

D


Rotarod
0~40 RPM
30 RPM
Latency to fall (s)
400
300
200
100
0
training
3d
15d
30d
Control
αS
MSC
Pole
Latency to descend (s)
80
60
40
20
0
*
Control
αS
MSC
At 30 days

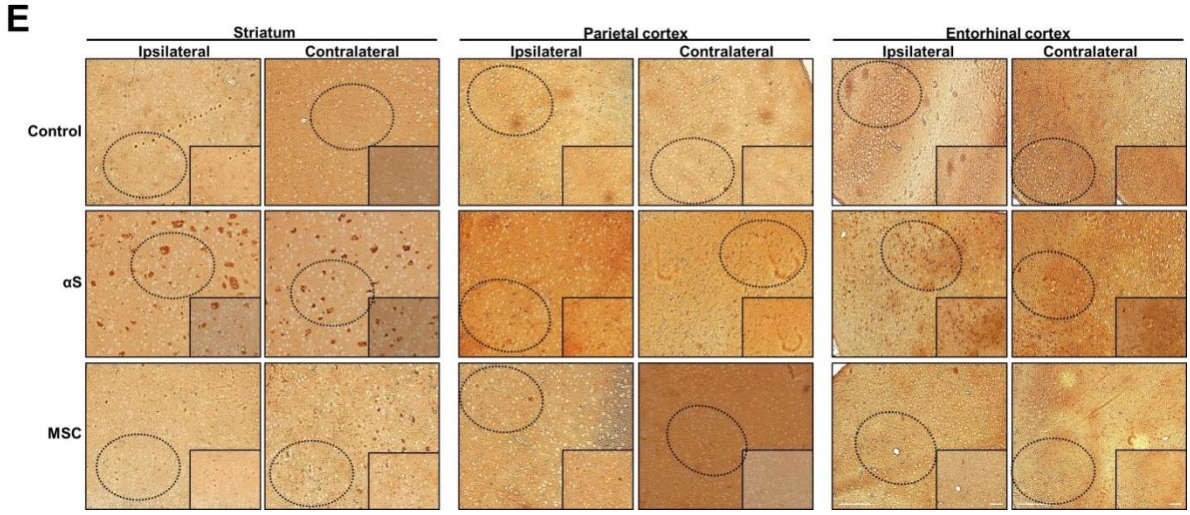


Figure 11. Long-term effects of MSCs on extracellular α-synuclein transmission. (A) The immunoreactivity of phosphorylated form of α-synuclein (p-αS) in the midbrain, showing that p-αS immunoreactivity was markedly attenuated MSC-treated animals compared to α-synuclein (αS)-inoculated animals. Scale bar, 10 μm. Arrowheads denote co-localization of Alexa 488 labeled αS with p-αS. (B) The immunoreactivity of p-αS in the TH-positive neurons of the SN was markedly attenuated MSC-treated animals compared to αS-inoculated animals. Scale bar, 10 μm. (C) The number of TH-positive neurons in the SN at 30 days after αS inoculation. Scale bar represents 10 μm. (D) Behavioral analysis, showing that αS inoculation led to progressive patterns of the latency to fall on the Rotarod test and increased latency to descend at 30 days after αS inoculation on fall test compared to the control group, whereas MSC treatment restored impaired motor coordination and balance on the Rotarod test and pole test (n=5, each group). (E) The distribution of p-αS accumulations in the striatum, parietal cortex, and entorhinal cortex, showing that MSC treatment in αS-inoculated animals significantly attenuated the immunoreactivity of p-αS in these areas. Scale bar, 10 μm. All data are presented as the menas ± s.e. $*P < 0.05$, $**P < 0.01$. I: ipsilateral, C: contralateral.

IV. DISCUSSION

The present study demonstrated that MSCs have inhibited propagation of α-synuclein via modulation of cell-to-cell transmissions by inhibiting NMDA receptor-mediated endocytosis, which led to a prosurvival effect on neurons with functional improvement of motor deficits in α-synuclein-enriched models. In addition, we found that Gal-1, soluble factor derived from MSCs, plays an important role in transmission control of extracellular α-synuclein in these models. Our data suggest that the property of MSCs in modulating propagation of extracellular α-synuclein may be applicable to future clinical strategies for treatment of patients with α-synucleinopathies.

α-Synuclein has a tendency to aggregate and accumulate, thus forming small intracellular aggregates, which could lead to an increase in cellular toxicity and cell death in various types of α-synucleinopathies.[29,30] Importantly, recent studies have provided evidence for cell-to-cell propagation of α-synuclein,[31,32] showing that α-synuclein and its aggregates are released from neuronal cells via exocytosis[31,33,34] and that neurons and glial cells have the ability to internalize extracellular α-synuclein aggregates through endocytosis.[35-37] In terms of their prion-like behavior, extracellular α-synuclein aggregates seem to play key roles in the pathogenesis and progression of α-synucleinopathies; therefore, treatment strategies focused on modulation of extracellular α-synuclein transmission would be clinically relevant. In this regard, the results of immunotherapy in animal models of Lewy body diseases are very suggestive, because antibodies against α-synuclein can modulate aggregated α-synuclein at several steps of accumulation and propagation.[38-42] Therefore, a therapeutic strategy to inhibit propagation of extracellular α-synuclein aggregates may be an important pharmacological target in disease-modifying treatment strategies for α-synucleinopathies.

Several studies have demonstrated that MSCs exert neuroprotective effects by secretion of neurotropic molecules that directly or indirectly can modulate neurodegenerative microenvironment.[43,44] Additionally, MSCs are known to secrete several molecules into the neural niche microenvironment,

which could promote endogenous neural repair.[45,46] Here, we found that MSCs have the ability to inhibit α-synuclein endocytosis and lead to inhibition of cell-to-cell transmission. In an α-synuclein-enriched cellular model, MSCs inhibited internalization of extracellular α-synuclein and blocked cell-to-cell transmission of α-synuclein in donor-acceptor cells model. As a result, MSCs significantly decreased the levels of internalized cytosolic α-synuclein and led to attenuation in α-synuclein-induced cell death. Specifically, we demonstrated that MSCs can inhibit CME of extracellular α-synuclein fibrils via modulation of interaction between α-synuclein and NMDA receptors. α-Synuclein treatment led to increased expression of clathrin and EEA1 with a concomitant decrease in immunoreactivity of surface NR1 and NR2A subunits. Meanwhile, MSC treatment significantly attenuated α-synuclein-induced expression of clathrin and EEA1 as well as interaction between α-synuclein and NMDA receptors. In an animal model of α-synuclein inoculation, MSC treatment markedly decreased the propagation of extracellular α-synuclein in regions of the brain at a distance from the inoculation site. When α-synuclein was inoculated in the cortex, MSC treatment markedly decreased the density of internalized α-synuclein and the pathogenic phosphorylated form of α-synuclein in in both ipsilateral and contralateral hemispheres. Moreover, MSC administration in α-synuclein-inoculated mice significantly decreased expression of clathrin, which was followed by decreased expression of EEA1and interaction between α-synuclein and NMDA receptors of NR1 and NR2A. Additionally, when α-synuclein was inoculated in the striatum, MSC treatment decreased phosphorylated form of α-synuclein expression in the midbrain at 30 days after inoculation. Consequently, modulation of extracellular α-synuclein propagation by MSCs led to exertion of a prosurvival effect on cortical neurons and nigral dopaminergic neurons with functional improvement of impaired motor coordination and balance against α-synuclein enriched environment.

Interestingly, we demonstrated in the present study that MSC-derived factor, Gal-1, could inhibit cell-to-cell transmission of α-synuclein via modulation of interaction between α-synuclein and NMDA

receptors. Gal-1, a galactose-binding lectin, is a multifunctional molecule involved in the regulation of cell adhesion, cell proliferation, and programmed cell death.[47] In nervous system, Gal-1 is involved in proliferation of neural stem cells, neritic outgrowth, and cellular adaptation of redox status[48,49] as well as regulation of glutamate toxicity via interaction with the NR1 subunit.[48] In an α-synuclein-enriched cellular model, Gal-1 treatment significantly decreased the levels of internalized cytosolic α-synuclein with concomitantly decreased clathrin and EEA1 expressions and increased NR1 expression. This modulatory effect of Gal-1 on cell-to-cell propagation of aggregated α-synuclein was further supported by Gal-1 siRNA treatment in vitro, showing that siRNA counteracted the inhibitory effect of MSCs on CME of α-synuclein via NMDA receptors and its associated prosurvival effects on neuronal cells. In vivo data showed that Gal-1 was co-expressed within MSCs injected intravenously, and Gal-1 treatment blocked CME of extracellular α-synuclein fibrils by inhibiting interaction of α-synuclein and surface NMDA receptors. In animals treated with enriched α-synuclein, this modulatory effect of Gal-1 on membrane trafficking of α-synuclein seemed to be comparable to those of MSCs, by showing that Gal-1 treatment markedly decreased the extent of inoculated α-synuclein aggregates, as well as expression of the pathological α-synuclein, compared to fresh medium. In this regard, the present study provides evidence that Gal-1 as MSC-derived soluble factor, can modulate the pathogenic microenvironments of extracellular α-synuclein via modulation of CME.

Although the exact mechanism contributing cell-to-cell transmission of α-synuclein is unknown, several possible routes mediated by direct penetration, fluid-phase or receptor-mediated endocytosis, the form of exosome, or nanotube have been suggested depending on the forms of α-synuclein. Of those, receptor-medicated endocytosis, which requires specific interactions between ligands and cell-surface receptors, seems to be a major mode of fibril internalization.[37] Along with evidence of α-synuclein participation in CME,[9] several studies suggested that higher molecular weight α-synuclein, such as aggregated fibrillar or oligomeric forms may be internalized through an endocytic pathway

via receptor.[9,24,25,50,51] Moreover, other studies have shown that the receptor-mediated endocytosis is also involved in amyloid-β transmission. Amyloid-β could decrease surface expression of NMDA receptors by promoting endocytosis of receptor proteins,[52,53] which is quite in accordance with the present result. Additionally, the laminin receptor acts as a central role in mediating the internalization of amyloid-β.[54] Taken together, the present data indicate that MSCs have a prosurvival effect on cortical and dopaminergic neurons against propagation of α-synuclein by modulating a major route of cell-to-cell transmission of toxic protein aggregates.

According to our in vitro data, the modulatory effect of MSCs on extracellular α-synuclein and their prosurvival effects on neuronal cells were not completely blocked by Gal-1 siRNA-treated CM. Additionally, even though MSCs significantly interacted with both NR1 and NR2A subunit, Gal-1 treatment did not show a significant interaction with surface NR2A subunit. These results suggest the existence of other MSC-derived soluble factors involved in aggregated α-synuclein transmission. Further studies are therefore required to identify MSC-derived small molecules responsible for extracellular α-synuclein modulation that would have clinical potential for the development of disease-modifying therapeutic strategies for α-synucleinopathies.

V. CONCLUSION

The present data indicated that MSCs exert neuroprotective properties through inhibition of cell-to-cell transmission of extracellular α-synuclein. In addition, Gal-1 may be the principal soluble factor released by MSCs responsible for modulation of extracellular α-synuclein. With advantages in clinical applications,[55,56] the use of MSCs or MSC-derived soluble factors as pharmacological modulators of α-synuclein propagation may be an effective therapeutic approach.

REFERENCES

1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha synuclein in Lewy bodies. Nature 1997;388:839-40.
2. Uversky VN, Li J, Fink AL. Evidence for a partially folded intermediate in alpha-synuclein fibril formation. J Biol Chem 2001;276:10737-44.
3. Borghi R, Marchese R, Negro A, Marinelli L, Forloni G, Zaccheo D, et al. Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. Neurosci Lett 2000;287:65-7.
4. El-Agnaf OM, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, et al. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J 2006;20:419-25.
5. Lee PH, Lee G, Park HJ, Bang OY, Joo IS, Huh K. The plasma alpha-synuclein levels in patients with Parkinson's diseaseand multiple system atrophy. J Neural Transm 2006; 113: 1435-9.
6. Brundin P, Melki R, Kopito R. Prion-like transmission of protein aggregates in neurodegenerative diseases. Nat Rev Mol Cell Biol 2010;11:301-7.
7. Hansen C, Angot E, Bergstrom AL, Steiner JA, Pieri L, Paul G, et al. alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J Clin Invest 2011;121:715-25.
8. Ben Gedalya T, Loeb V, Israeli E, Altschuler Y, Selkoe DJ, Sharon R. Alpha-synuclein and polyunsaturated fatty acids promote clathrin-mediated endocytosis and synaptic vesicle recycling. Traffic 2009;10:218-34.
9. Cheng F, Li X, Li Y, Wang C, Wang T, Liu G, et al. alpha-Synuclein promotes clathrin-mediated NMDA receptor endocytosis and attenuates NMDA-induced dopaminergic cell death. J Neurochem 2011;119:815-25.

10. van Dam EM, Stoorvogel W. Dynamin-dependent transferrin receptor recycling byendosome-derived clathrin-coated vesicles. Mol Biol Cell 2002;13:169-82.

11. Carroll RC, Zukin RS. NMDA-receptor trafficking and targeting: implications for synaptic transmission and plasticity. Trends Neurosci 2002;25:571-7.

12. Lavezzari G, McCallum J, Dewey CM, Roche KW. Subunit-specific regulation of NMDA receptor endocytosis. J Neurosci 2004;24:6383-91.

13. Lavezzari G, McCallum J, Lee R, Roche KW. Differential binding of the AP-2adaptor complex and PSD-95 to the C-terminus of the NMDA receptor subunit NR2B regulates surface expression. Neuropharmacol 2003;45:729-37.

14. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physio1998;176:57-66.

15. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006; 98:1076-84.

16. Kim HS, Choi DY, Yun SJ, Choi SM, Kang JW, Jung JW, et al. Proteomic analysis of microvesicles derived from human mesenchymal stem cells. J Proteome Res 2012;11:839-49.

17. Kim YJ, Park HJ, Lee G, Bang OY, Ahn YH, Joe E, et al. Neuroprotective effects of human mesenchymal stem cells on dopaminergic neurons through anti-inflammatory action. Glia 2009;57:13-23.

18. Park HJ, Shin JY, Lee BR, Kim HO, Lee PH. Mesenchymal stem cells augment neurogenesis in the subventricular zone and enhance differentiation of neural precursor cells into dopaminergic neurons in the substantia nigra of a parkinsonian model Cell Transplant 2012; 21:1629-40.

19. Park HJ, Bang G, Lee BR, Kim HO, Lee PH. Neuroprotective effect of human mesenchymal stem cells in an animal model of double toxin-induced multiple system atrophy parkinsonism.

Cell Transplant 2011;20:827-35.

20. Park HJ, Shin JY, Kim HN, Oh SH, Lee PH. Neuroprotective effects of mesenchymal stem cells through autophagy modulation in a parkinsonian model. Neurobiol Aging 2014;35:1920-8.

21. Shin JY, Park HJ, Kim HN, Oh SH, Bae JS, Ha HJ, et al. Mesenchymal stem cells enhance autophagy and increase beta-amyloid clearance in Alzheimer disease models. Autophagy 2014;10:32-44.

22. Lopes FM, Schroder R, da Frota ML, Jr., Zanotto-Filho A, Muller CB, et al. Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res 2010;1337:85-94.

23. Rabilloud T, Kieffer S, Procaccio V, Louwagie M, Courchesne PL, Patterson SD, et al. Two-dimensional electrophoresis of human placental mitochondria and protein identification by mass spectrometry: toward a human mitochondrial proteome. Electrophoresis 1998;19:1006-14.

24. Krueger RC, Jr., Schwartz NB. An improved method of sequential alcian blue and ammoniacal silver staining of chondroitin sulfate proteoglycan in polyacrylamide gels. Anal Biochem 1987;167:295-300.

25. Fernandez J, Gharahdaghi F, Mische SM. Routine identification of proteins from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels or polyvinyl difluoride membranes using matrix assisted laser desorption/ionization-time offlight-mass spectrometry (MALDI-TOF-MS). Electrophoresis 1998;19:1036-45.

26. Datki Z, Juhasz A, Galfi M, Soos K, Papp R, Zadori D, et al. Method for measuring neurotoxicity of aggregating polypeptides with the MTT assay on differentiated neuroblastoma cells. Brain Res Bull 2003;62:223-9.

27. Matsuura K, Kabuto H, Makino H, Ogawa N. Pole test is a useful method for evaluating the

mouse movement disorder caused by striatal dopamine depletion. J Neurosci Methods 1997; 73:45-8

28. West MJ. New stereological methods for counting neurons. Neurobiol Aging 1993;14:275-85

29. Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med 1998;4:1318-20.

30. Uversky VN, Lee HJ, Li J, Fink AL, Lee SJ. Stabilization of partially folded conformation during alpha-synuclein oligomerization in both purified and cytosolic preparations. J Biol Chem 2001;276:43495-8.

31. Lee HJ, Bae EJ, Lee SJ. Extracellular alpha--synuclein-a novel and crucial factor in Lewy body diseases. Nat Rev Neurol 2014;10:92-98.

32. Lee, S.J., Desplats, P., Sigurdson, C., Tsigelny, I. & Masliah, E. Cell-to-cell transmission of non-prion protein aggregates. Nat Rev Neurol 2010;6:702-6.

33. Lee HJ, Patel S, Lee SJ. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. J Neurosci 2005;25:6016-24.

34. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 2008;14:501-3.

35. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, et al. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 2010;285:9262-72.

36. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U. S. A. 2009;106:13010-5.

37. Guo JL, Lee VM. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat Med 2014;20:130-8.

38. Bae EJ, Lee HJ, Rockenstein E, Ho DH, Park EB, Yang NY, et al. Antibody-aided clearance of extracellular alpha-synuclein prevents cell-to-cell aggregate transmission. J Neurosci 2012; 32:13454-69.

39. Fagerqvist T, Lindstrom V, Nordstrom E, Lord A, Tucker SM, Su X, et al. Monoclonal antibodies selective for alpha-synuclein oligomers/protofibrils recognize brain pathology in Lewy body disorders and alpha-synuclein transgenic mice with the disease-causing A30P mutation. J Neurochem. 2013;126:131-44.

40. Masliah E, Rockenstein E, Mante M, Crews L, Spencer B, Adame A, et al. Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. PloS one 2011;6:e19338

41. Valera E, Masliah E. Immunotherapy for neurodegenerative diseases: focus on alpha-synucleinopathies. Pharmacol Ther 2013;138:311-22.

42. Tran HT, Chung CH, Iba M, Zhang B, Trojanowski JQ, Luk KC, et al. Alpha-synuclein immunotherapy blocks uptake and templated propagation of misfolded alpha-synuclein and neurodegeneration. Cell Rep 2014;7:2054-65.

43. Mathieu P, Roca V, Gamba C, Del Pozo A, Pitossi F. Neuroprotective effectsof human umbilical cord mesenchymal stromal cells in an immunocompetent animal model of Parkinson's disease. J Neuroimmunol 2012;246:43-50.

44. Paul G, Anisimov SV. The secretome of mesenchymal stem cells: potential implications for neuroregeneration. Biochimie 2013;95:2246-56.

45. Crigler L, Robey RC, Asawachaicharn A, Gaupp D. Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp Neurol 2006;198:54-64.

46. Park HJ, Lee PH, Bang OY, Lee G, Ahn YH. Mesenchymal stem cells therapy exerts neuroprotection in a progressive animal model of Parkinson's disease. J Neurochem

2008;107:141-51.

47. Perillo NL, Marcus ME, Baum LG. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. Mol Med 1998;76:402-12.

48. Lekishvili T, Hesketh S, Brazier MW, Brown DR. Mouse galectin-1 inhibits the toxicity of glutamate by modifying NR1 NMDA receptor expression. Eur J Neurosci 2006;24:3017-25.

49. Miura T, Takahashi M, Horie H, Kurushima H, Tsuchimoto D, Sakumi K, et al. Galectin-1beta, a natural monomeric form of galectin-1 lacking its sixamino-terminal residues promotes axonal regeneration but not cell death. Cell Deathb Differ 2004;11:1076-83.

50. Lee HJ, Suk JE, Bae EJ, Lee JH, Paik SR, Lee SJ. Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. Int J Biochem Cell Biol 2008;40: 1835-49.

51. Chai YJ, Kim D, Park J, Zhao H, Lee SJ, Chang S. The secreted oligomeric form of alpha-synuclein affects multiple steps of membrane trafficking. FEBS Lett 2013;587: 452-9.

52. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 2005;8:1051-8

53. Sakono M, Zako T. Amyloid oligomers: formation and toxicity of Abeta oligomers. FEBS J 2010;277:1348-58.

54. Da Costa Dias B, Jovanovic K, Gonsalves D, Moodley K, Reusch U, Knackmuss S, et al. The 37kDa/67kDa laminin receptor acts as a receptor for Abeta42 internalization. Sci Rep 2014; 4: 5556.55.

56. Lee PH, Lee JE, Kim HS, Song SK, Lee HS, Nam HS, et al. A randomized trial of mesenchymal stem cells in multiple system atrophy. Ann Neurol 2012;72:32-40.

ABSTRACT (IN KOREAN)

파킨슨병 모델에서 중간엽 줄기세포가 클라트린 매개 엔도사이토시스를 조절함으로써 알파시뉴클레인의 세포 간 전달 억제

<지도교수 이 필 휴>

연세대학교 대학원 의과학과

김 하 나

알파시뉴클레인은 세포로부터 방출된 후, 세포 간 전달에 의해 뇌의 한 영역에서 다른 영역으로 전달될 수 있다고 보고된 바 있다. 세포 외 알파시뉴클레인 응집체는 프리온과 유사한 거동을 보이므로 알파시뉴클레인 병증의 발병과 전파에 중요한 역할을 하는 것으로 생각된다. 중간엽 줄기세포(mesenchymal stem cell; 이하, 'MSC'라 한다)는 신경염증 조절, 세포 생존과 관련된 신호, 신경발생의 증가, 오토파지 조절과 같은 다양한 메커니즘을 통하여 신경보호 효과를 가지는 사이트토로픽 팩터를 분비한다. 알파시뉴클레인을 처리한 모델을 사용하여, MSC 가 N-메틸-D-아스파르테이트 (N-methyl-D-aspartate, 이하 'NMDA'라 한다) 수용체 사이의 상호작용을 조절하여 알파시뉴클레인의 클라트린 매개 엔도사이토시스를 억제함으로써 알파시뉴클레인의 세포 간 전달을 억제하여 운동기능의 향상과 함께 프로서바이벌 효과로 이어짐을 확인하였다. 알파시뉴클레인을 주입한 동물모델에서, MSC 혹은 MSC 조정배지는 알파시뉴클레인의 접종 위치인 동측 부위에서 서측 부위로의 알파시뉴클레인의 전달이 억제됨과 동시에 알파시뉴클레인 응집체의 크기가 감소함을 증명하였다. 또한, MSC 에서 유래된 갈렉틴-1 을 유효성분으로 포함하여 세포 외 알파시뉴클레인의 세포 간 전달을 억제하는데

중요한 역할을 함을 증명한다. 이에, 본 연구는 MSC 가 세포 외 알파시뉴클레인의 세포간 전달을 억제함으로써 신경 보호 특성을 가짐을 밝힌다.

핵심되는 말 : 중간엽줄기세포, 알파시뉴클레인, 전달, 파킨슨병