Biochemical and Biophysical Research Communications 464 (2015) 526–533

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Rejuvenation of MPTP-induced human neural precursor cell senescence by activating autophagy

Liang Zhu [a, 1], Chuanming Dong [a, b, 1], Chenxi Sun [a], Rongjie Ma [a], Danjing Yang [a], Hongwen Zhu [c, **], Jun Xu [a, *]

[a] East Hospital, Tongji University School of Medicine, Shanghai, China
[b] Department of Anatomy and Neurobiology, The Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong, China
[c] Tianjin Hospital, Tianjin Academy of Integrative Medicine, Tianjin, China

ARTICLE INFO

Article history:
Received 29 June 2015
Accepted 30 June 2015
Available online 6 July 2015

Keywords:
Human neural precursor cell
Aging
Autophagy
Cellular senescence
MPTP

ABSTRACT

Aging of neural stem cell, which can affect brain homeostasis, may be caused by many cellular mechanisms. Autophagy dysfunction was found in aged and neurodegenerative brains. However, little is known about the relationship between autophagy and human neural stem cell (hNSC) aging. The present study used 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) to treat neural precursor cells (NPCs) derived from human embryonic stem cell (hESC) line H9 and investigate related molecular mechanisms involved in this process. MPTP-treated NPCs were found to undergo premature senescence [determined by increased senescence-associated-β-galactosidase (SA-β-gal) activity, elevated intracellular reactive oxygen species level, and decreased proliferation] and were associated with impaired autophagy. Additionally, the cellular senescence phenotypes were manifested at the molecular level by a significant increase in p21 and p53 expression, a decrease in SOD2 expression, and a decrease in expression of some key autophagy-related genes such as Atg5, Atg7, Atg12, and Beclin 1. Furthermore, we found that the senescence-like phenotype of MPTP-treated hNPCs was rejuvenated through treatment with a well-known autophagy enhancer rapamycin, which was blocked by suppression of essential autophagy gene Beclin 1. Taken together, these findings reveal the critical role of autophagy in the process of hNSC aging, and this process can be reversed by activating autophagy.

1. Introduction

Human neural stem cells (hNSCs) are important in maintaining human brain homeostasis. New neurons are continuously generated form NSCs, which are important for cognitive functions [1]. Aging is accompanied by progressive decline in the proliferation of NSCs and cellular senescence, leading to marked age-related cognitive deficits such as olfactory dysfunction, spatial memory deficits, and neurodegenerative disorders [2,3].

The *in vitro* differentiation of human embryonic stem cells (hESCs) overcomes the difficulties in obtaining human neural precursor cells (NPCs) and facilitates the study of aging process of hNSCs [4]. The current study explores the cellular senescence of human embryonic stem cell (hESC)-derived NPCs and investigates related molecular mechanisms involved in this process.

1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is used extensively as an experimental model for Parkinson's disease [5]. 1-Methyl-4-phenylpyridine ion (MPP^+), a metabolite of MPTP, is believed to concentrate in mitochondria where it inhibits complex I of the electron transport system, leading to the generation of ROS and mitochondrial dysfunction that can contribute to aging [6]. L'Episcopo et al. found that the MPTP treatment can impair proliferation and neuron differentiation properties of NPCs isolated from the mice subventricular zone (SVZ), which decrease with age [7]. However, they did not demonstrate whether the loss of proliferative and neurogenic potential after MPTP administration is

* Corresponding author. East Hospital, Tongji University School of Medicine, 150 Jimo Road, Shanghai 200120, China.
** Corresponding author. Tianjin Academy of Integrative Medicine, Tianjin Hospital, Tianjin 300211, China.
E-mail addresses: hongwen_zhu@hotmail.com (H. Zhu), xunymc2000@yahoo.com (J. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.06.174
0006-291X/

caused by cellular senescence of NPCs. This study aimed to investigate whether MPTP can induce the cellular senescence of human NPCs as an aging model and study the related mechanism involved in it.

Autophagy is an important degradation process, which can remove the damaged mitochondria and aggregated proteins [8,9]. Various studies found that the expression of autophagy-related genes and the efficiency of autophagic process decline with aging [10]. The inhibition of autophagy could accelerate premature aging, whereas the enhancement of autophagy might delay aging and extend lifespan [11]. Rapamycin, a pharmacological autophagy enhancer, has been shown to prolong life and delay the onset of age-related diseases in animals [12]. However, no effort has yet been documented concerning whether autophagy is contributing to the aging of hNSCs.

In this study, we used MPTP to treat the hNPCs derived from hESC line H9, mimic the aging process of the hNSCs, and explored the molecular mechanism of hNSC aging. We detected that after MPTP treatment, NPCs showed aging phenotype manifested by augmented senescence-associated-β-galactosidase (SA-β-gal) staining, decreased proliferation, elevated ROS level, which is associated with inhibited autophagy. Interestingly, the senescence-like phenotype of MPTP-treated hNPCs was restored through treatment with rapamycin, which was blocked when autophagy-related gene Beclin 1 was suppressed.

2. Materials and methods

2.1. Human embryonic stem cell culture and neural induction

Human ES cell line (H9) with a stable normal (46XX) karyotype were plated onto a feeder layer of irradiated mouse embryonic fibroblasts in ES cell medium containing 80% DMEM/F12, 20% knockout serum replacement, 0.1 mM β-mercaptoethanol, 1 mM glutamine, and 1% nonessential amino acids, supplemented with 10 ng/ml bFGF. The hESCs were fed every day until ready to passage. To passage hESCs, cells were incubated with 1 mg/ml collagenase IV in DMEM/F12 for about 15–30 min at 37 °C. Then the clonies were collected, gently dissociated into small clusters and plated in fresh ES cell medium.

The induction of neural precursor cells was based on a previous report [13]. Briefly, after digestion and trituration, cells were resuspended in ES cell medium without FGF-2 for 6 days as floating EBs. Then medium was then switched to N2 medium consisting of DMEM/F12, 1% N2 supplement, 1% nonessential amino acids, 2 μg/ml heparin, 10 ng/ml bFGF for 10 days. To separate the rosette clusters from the differentiating EBs, the cells were incubated with 0.1 mg/ml dispase at 37 °C for 15–20 min. Then the rosette clumps were isolated, gently triturated and plated in fresh N2 medium as neural precursors. Culture was fed every 2 or 3 days. Cells were subcultured once a week by triturating the neurospheres after digestion with dispase. For adhesion culture, the hNPCs were cultured in matrigel-coated plates.

To analyze the differentiation potential, hNPCs were cultured on poly-ornithine/laminin-coated plates in N2 medium without bFGF for 10 days. Medium were changed every 3 days.

2.2. Cell senescence assay

Cellular senescence was determined using the SA-β-gal staining Kit (Genmed Scientifics). Briefly, after 12–16 h incubation at 37 °C, the stained cells were captured with an optic microscope with quantifications obtained from at least 10 fields.

2.3. Reactive oxygen species measurement

To measure intracellular ROS, we loaded cells with DCFH-DA (Genmed Scientifics) at 37 °C for 20 min and then moved to the Nikon E800 fluorescence microscope, intracellular fluorescence intensity were analyzed in ImageJ. For dyes fluorescence was excited with 488 nm laser and observed in red spectral region (525–535 nm). Experiments were performed in triplicates from three independent trials.

2.4. Cell counting kit-8 (CCK8) assay

For the cell viability assessment, hNPCs derived from human embryonic stem cells were plated onto 96-well plates (about 3×10^3 cells/well). Twenty-four hours after seeding the cells, each well was treated with MPTP for 24 h, with the final concentration of 200, 400, 600, 800, and 1000 μM. At the end of each time point, 10 μL WST-8 solution (Dojindo Laboratories, Japan) was added to each well, and the plates were incubated for an additional 2.5 h at 37 °C. The absorbance of each plate at 450 nm represented a direct correlation with the cell number in this analysis and was measured using a standard microplate reader (Thermo, Varioskan Flash).

For the cell proliferation assays, hNPCs were cultured in 96-well plates at 3×10^3 cells per well with growth culture medium. Twenty-four hours later, culture was treated with MPTP. Cells were incubated with the MPTP at 37 °C for 12, 24, 36, 48hrs. At the end of each time point, 10 μl WST-8 solution was added to each well, and the plates were incubated for an additional 2.5 h at 37 °C. The proliferation of hNPCs was measured by microplate reader scanning at 450 nm.

2.5. MPTP treatment

Cells were seeded at 1×10^4 cells/ml in 24-well plates (Corning Incorporated, USA), treated with MPTP (Sigma, St. Louis, MO, USA) at a concentration of 400 μM, and incubated at 37 °C until the collection time points.

2.6. Rapamycin treatment

Cells were seeded at 1×10^4 cells/mL in 24-well plates, pretreated with rapamycin (Absin Bioscience Inc., China) at a concentration of 0.04 μM, and then exposed to 400 μM MPTP followed by incubation at 37 °C until the collection time points.

2.7. Transfection of cells with Beclin 1 siRNA

Cells were transiently transfected with small interference RNA (siRNA) of Beclin 1 (sense, GGAGCCAUUUAUUGAAACUTT; antisense, AGUUUCAAUAAAUGGCUCCTT) or control nonspecific siRNA (sense, UUCUCCGAACGUGUCACGUTT; antisense, ACGUGACACGUU CGGAGAATT) using FuGENE HD Transfection Reagent (Promega). 48 h after transfection, cells were exposed to various treatments as specifically indicated.

2.8. Immunocytochemistry

Cells grown on coverslips were fixed in 4% paraformaldehyde for 15 min at room temperature, permeabilized with 0.3% Triton X-100 in PBS for 10 min, and blocked in 3% normal donkey serum in PBS for 2 h at room temperature. Primary antibodies were diluted in 3% normal donkey serum in PBS and applied at 4 °C overnight. The primary antibodies used in these experiments were as follow: Nestin (Sigma; 1:200), Ki67 (Thermo; 1:500), GFAP (millpore; 1:3000), Tuj1 (covance; 1:10000), Sox2 (Santa Cruz; 1:1000), after

rinsing in PBS three times and incubating for 2 h with CF488 and CF543 (Biotium; 1:1000), coverslips were washed three times, cell nuclei were stained with DAPI (Sigma; 1:1000). Images were acquired on a Leica TCS SP2 confocal fluorescence microscope.

2.9. Real-time quantitative PCR

For QRT-PCR, total RNA was reverse-transcribed using SuperScript III (Invitrogen). All reactions were performed using SYBR® Green PCR Core reagents (Applied Biosystems). Primers were designed using Primer Express software (Applied Biosystems) and experimentally validated. Sequences for primers used are as follows:

Gene	QRT-PCR forward primer	QRT-PCR reverse primer
p21	TACTTCCTCTGCCCTGCTGC	GCTGGTCTGCCTCCGTTTT
P53	TTCAGGCTTATGGAAACTAC	AGAAGGGACAAAAGATGACA
Atg5	AGAAGCTGTTTCGTCCTGTGG	AGGTGTTTCCAACATTGGCTC
Atg7	ATGATCCCTGTAACTTAGCCCA	CACGGAAGCAAACAACTTCAAC
Atg12	TAGAGCGAACACGAACCATCC	CACTGCCAAAACACTCATAGAGA
Beclin 1	ACCTCAGCCGAAGACTGAAG	AACAGCGTTTGTAGTTCTGACA
GAPDH	GGTGAAGGTCGGTGTGAACG	CTCGCTCCTGGAAGATGGTG

The expression of each gene was defined from the threshold cycle (C_t), and relative expression levels were calculated by using the △△CT method after normalization with reference to expression of the housekeeping gene GAPDH. Results are means from three individual experiments.

2.10. Western blotting

Protein samples were prepared from human neural precursor cells treated with or without MPTP. Protein concentrations were measured by the BCA protein assay kit (Thermo Scientific), and cell lysates were applied to 8% SDS-polyacrylamide gels (Bio-Rad), transferred to a PVDF membrane (Millipore). Primary antibodies were β-actin (Sigma), p53 (Abcam), p21 (Proteintech), Beclin 1 (ABclonal Technology), SOD2 (ABclonal Technology), β-actin was included as a loading control.

2.11. Statistical analysis

Data were analyzed by Student's t-test. *P* values of <0.05 were considered significant.

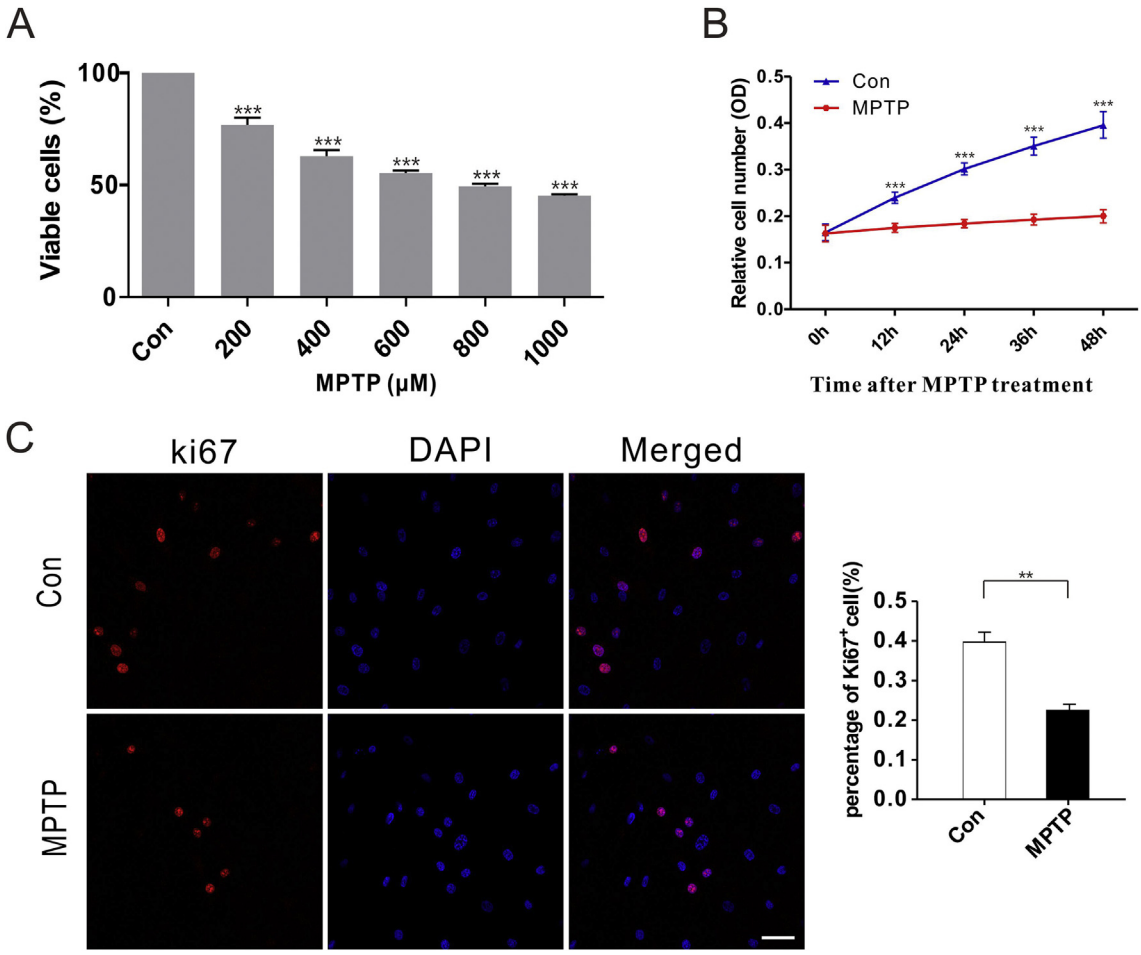

Fig. 1. hESC-derived NPCs showed an obvious decrease in proliferation after MPTP treatment. (A) hESC-derived neural precursor cells were treated with MPTP at indicated concentrations for 24 h. The percentage of viable cells was evaluated in 2.5 h after treatment using CCK-8 assay. Results are shown as a percent of control. (B) Effect of MPTP on cell proliferation was analyzed at 12, 24, 36, and 48 h. The relative cell number (value of OD) dramatically decreased after 400 μM MPTP treatment compared to the control. (C) Proliferation capacity from control and MPTP-treated NPCs was determined by ki67 staining. Graph shows the percentage of ki67$^+$ cells. Scale bars, 50 μm. Data are expressed as Mean ± SEM from three independent experiments, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Generation of hESC-derived neural precursor cells

Highly enriched cultures of NPC were generated from hESCs according to the protocols described [13]. During subsequential subculturing, the NPC cultures maintained their stem cell characteristics and stained positively for Nestin and SOX2 as adhered monolayer cells (Fig. S1A). The NPCs can also undergo differentiation toward neurons that expressed Tuj1, astrocytes that expressed GFAP (Fig. S1B and C).

3.2. Cell viability after MPTP treatment

MPTP treatment can cause mitochondrial dysfunction, which is an important inducer of aging. To find out if MPTP treatment can induce human NPC senescence, it is necessary to assess hNPCs viability after exposing these to MPTP to choose a suitable concentration required for further experiments.

NSCs were treated for 24 h with varying concentrations of MPTP (from 200 μM to 1000 μM). Cell viability was evaluated by CCK8 assay, which is a broad indicator of cellular activity. As shown in Fig. 1A, MPTP affected the cell viability in a dose-dependent manner. The LD50 value characterizing cell viability corresponded to 800 μM for hNPCs.

3.3. MPTP treatment-induced senescence-like state in hESC-derived NPCs

First, the cell proliferation of NPCs was analyzed by the CCK8 assay at 12, 24, 36, and 48 h (Fig. 1B). The treatment of hESCs with 400 μM MPTP induced a dramatic decrease in the relative cell number (value of OD) compared to the control in a time-dependent manner ($P < 0.001$). After this, the cell proliferation rate was analyzed using an antibody against Ki67, a marker for proliferating cells. Consistently, the percentage of Ki67$^+$ NPCs was smaller after MPTP treatment (Fig. 1C).

It is believed that MPTP inhibits the electron transport system leading to the generation of ROS, which is also proved to be a major

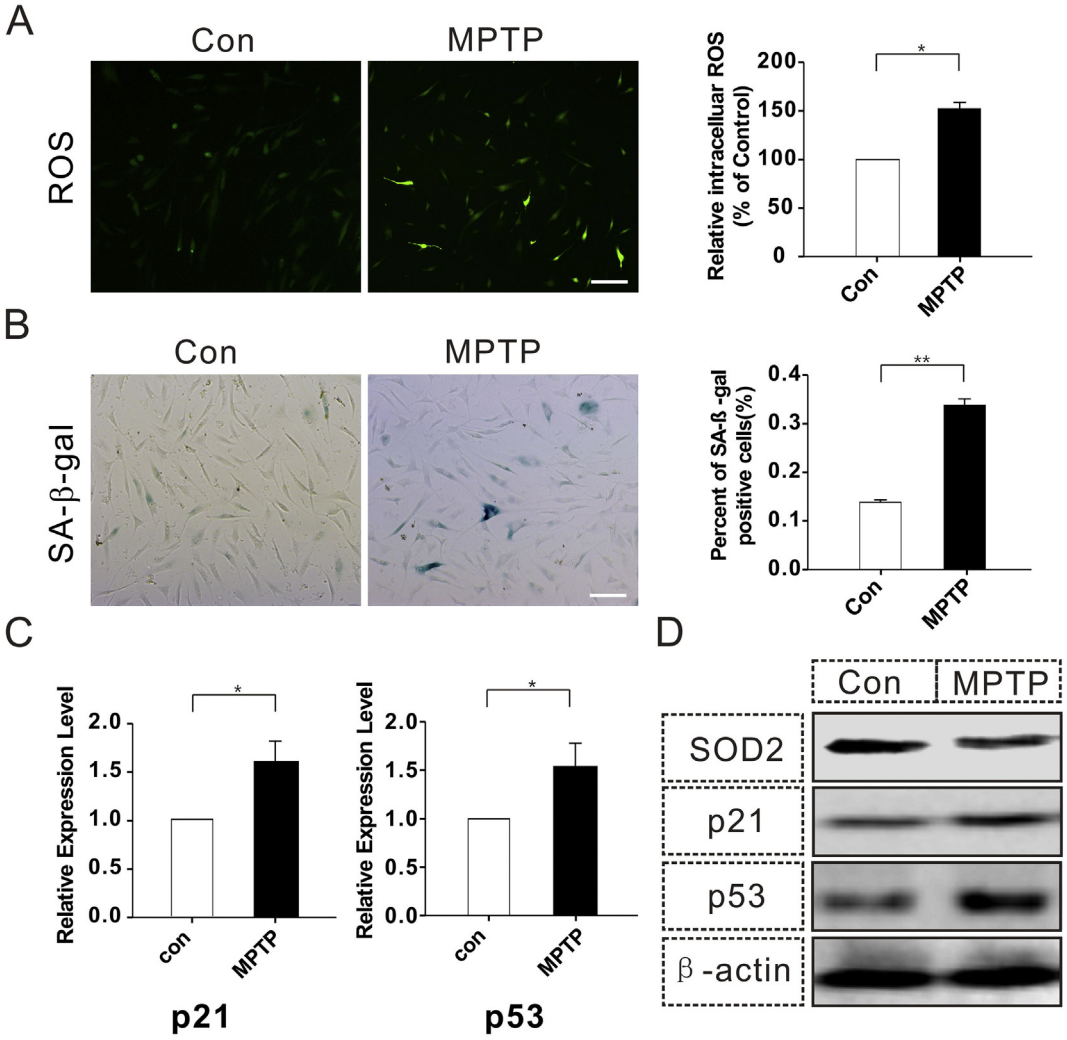

Fig. 2. MPTP treatment induced a senescence-like state in hESC-derived NPCs. (A) Intracellular ROS level was detected using dihydroethidium fluorescence. Graph shows the ROS level normalized to that of control cells. Scale bars, 100 μm. (B) Images of SA-β-gal$^+$ NSCs with or without MPTP treatment. Graph shows the percentage of SA-β-gal$^+$ NPCs. Scale bars, 100 μm. (C) QRT-PCR analysis of p21 and p53 expression from control and MPTP-treated NPCs normalized by GAPDH. (D) Western blotting analysis of SOD2, p21, and p53 expression from control and MPTP-treated NPCs. Data are expressed as Mean ± SEM from three independent experiments, $^*P < 0.05$, $^{**}P < 0.01$.

factor for aging [14]. Consistent with the research finding, the MPTP-treated NPCs augmented the ROS level in hESC-derived NPCs than in the control cells (Fig. 2A); decrease in the expression level of SOD2—a key gene coding for enzyme involved in the elimination of ROS—was also observed (Fig. 2D).

A well-known senescent-associated biomarker (SA-β-gal) was assessed to examine whether NPCs after treatment with MPTP could undergo cell senescence. MPTP treatment induced augmented β-galactosidase expression. The percentage of SA-β-gal^{+} NPCs increased from 8.39% in the control group to 26.63% in the MPTP-treated group (Fig. 2B).

p53/p21 pathway is believed to be related to cell cycle arrest and cell senescence. To find out whether p53/p21 pathway could be involved in the regulation of MPTP-treated senescence of NPCs, the expression levels of p21 and p53 were determined by QRT-PCR and Western blot. The results showed that 400 μM MPTP promoted a significant elevation in mRNA (Fig. 2C) and protein (Fig. 2D) expression of p21 and p53.

3.4. Impaired autophagy of hESC-derived NPCs induced by MPTP treatment

It is reported that autophagy dysfunction relates to aging; therefore, the mechanistic correlation between autophagy and MPTP-induced NPC senescence was investigated. The expression of some major autophagy genes was examined, which included Atg5,

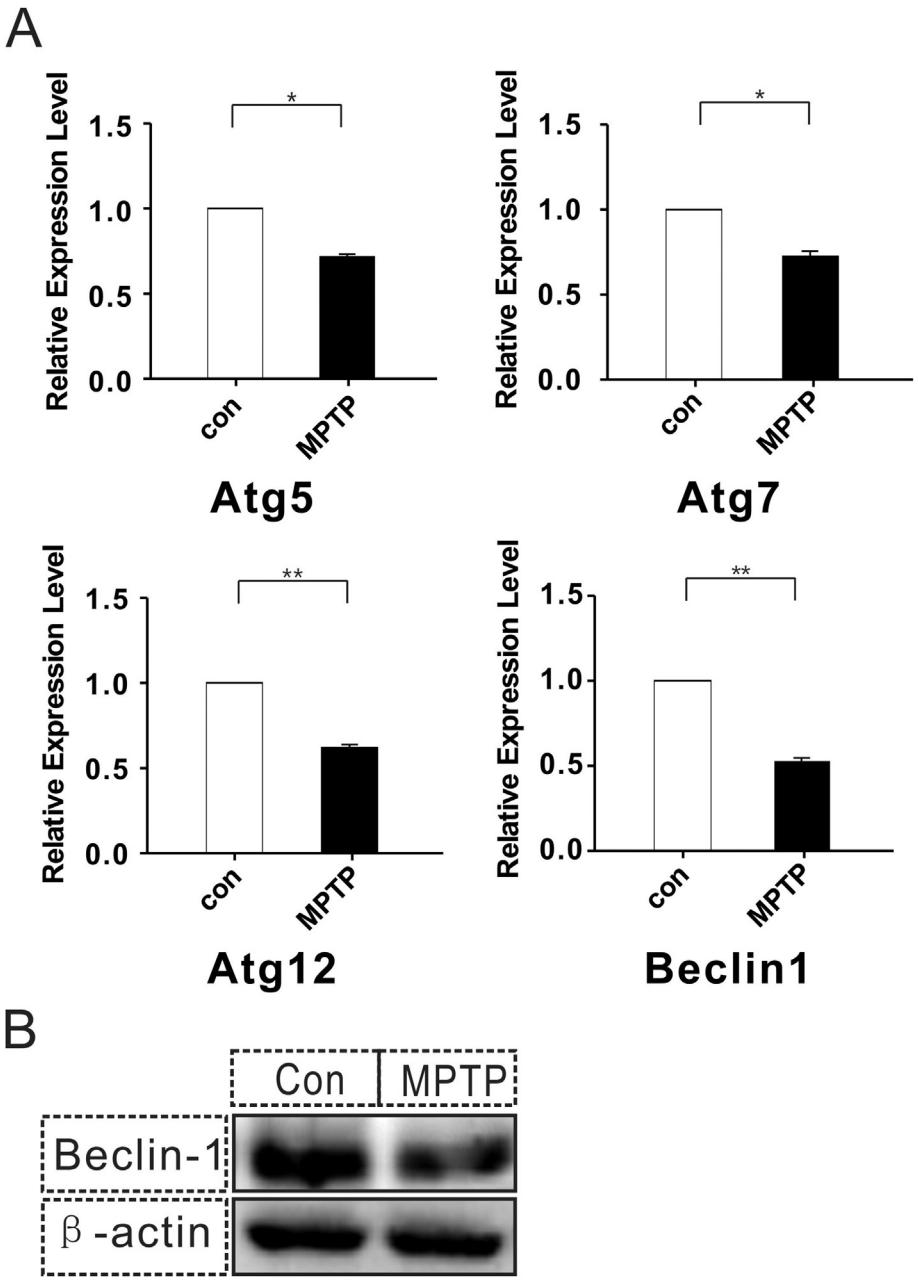

Fig. 3. Impaired autophagy of hESC-derived NPCs induced by MPTP treatment. (A) QRT-PCR analysis of Atg5, Atg7, Atg12, and Beclin 1 expression from control and MPTP-treated NPCs normalized by GAPDH. (B) Western blotting analysis of Beclin 1 expression from control and MPTP-treated NPCs. Data are expressed as Mean ± SEM from three independent experiments, $^{*}P < 0.05$, $^{**}P < 0.01$.

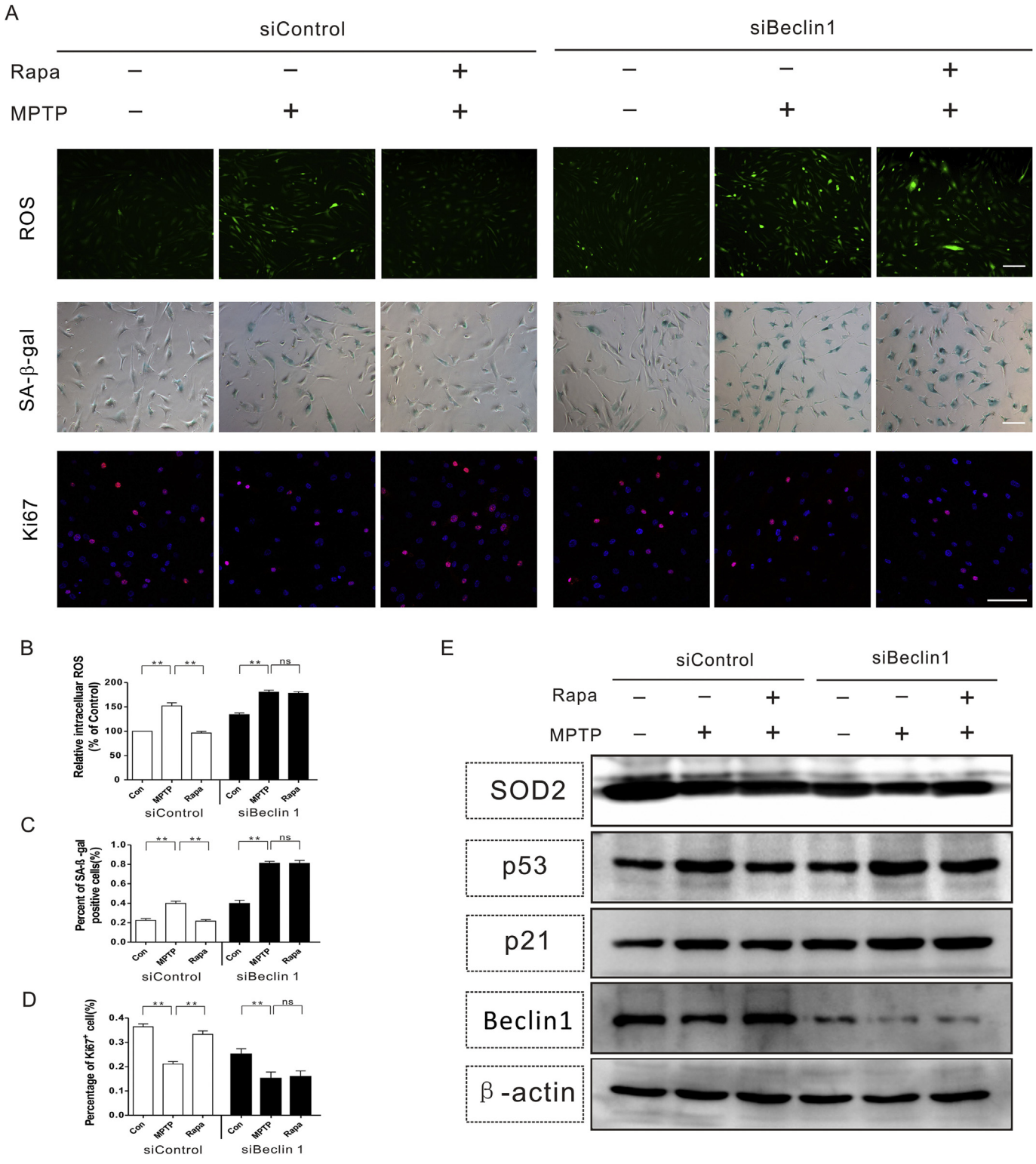

Fig. 4. Rapamycin can rejuvenate hESC-derived NPCs induced by MPTP treatment through autophagy induction. hESC-derived NPCs were transfected with Beclin 1 siRNA (100 nM) or negative control siRNA for 48 h followed by either left untreated or treated with MPTP or rapamycin, and the following assessments were made: (A) intracellular ROS level, SA-β-gal staining, Ki67 immunofluorescence. Scale bars, 100 μm. (B) Showed the statistical graph of relative intracelluar ROS (% of Control), (C) Showed the statistical graph of percent of SA-β-gal positive cells (%), (D) Showed the statistical graph of percentage of $Ki67^+$ cell (%), (E) SOD2, p53, p21 and Beclin 1 protein levels measured by Western blotting analysis. Data are expressed as Mean ± SEM from three independent experiments, $^{**}P < 0.01$.

Atg7, Atg12, and Beclin 1. MPTP treatment decreased the expression of Atg5, Atg7, Atg12, and Beclin 1 as unraveled by QRT-PCR (Fig. 3A) and Western blot (Fig. 3B) analyses.

3.5. Autophagy stimulation can rejuvenate hESC-derived NPCs induced by MPTP treatment

Whether enhanced autophagy would be able to abrogate MPTP-induced NPCs senescence was examined to extrapolate the molecular mechanisms of hESC-derived NPCs senescence. Experimental activation of autophagy was performed by rapamycin, a wildly used pharmacological autophagy inducer, at a concentration of 0.04 μM followed by 400 μM MPTP treatment. First, ROS level and SA-β-gal staining were measured, as described in Section 3.3. Rapamycin administration was found to significantly decrease ROS levels and the percentage of SA-β-gal$^+$ NPCs (Fig. 4A–C), and the proliferation of MPTP-treated NPCs was improved, which was manifested by Ki67 immunofluorescence after rapamycin treatment (Fig. 4A and D).

Additionally, we found that the attenuation of MPTP-induced senescence-like state of NPCs after rapamycin treatment was blocked when the essential autophagy gene Beclin 1 was suppressed by siRNA transfection (Fig. 4A–C). Also the MPTP–induced senescence phenotype was aggravated after Beclin 1 suppression (Fig. 4A–C).

The levels of p21, p53, and SOD2 were compared after various treatments. The results of Western blotting (see Fig 4E) demonstrated that the rapamycin administration diminished the levels of p21 and p53 and increased the level of SOD2 in MPTP-induced human NPCs. Meanwhile, the restoration of P21, P53, SOD2 after rapamycin exposure was obviously abolished when Beclin 1 was suppressed. These data establish the functional significance of enhanced autophagy for rejuvenating human NPCs.

4. Discussion

The findings of this study showed that hESC-derived NPCs are able to undergo premature senescence induced with 400 μM MPTP. This is evidenced by SA-β-gal positivity, decreased proliferation, and elevated ROS level. The MPTP-induced NPC senescence was accompanied by a significant elevation in senescence gene expression such as p21 and p53 and decreased the level of SOD2. Furthermore, The MPTP-induced NPC senescence was accompanied by a significant reduction in some key autophagy-related gene expression. Activating autophagy through rapamycin increased NPC proliferation and rejuvenated the senescence-like phenotype of MPTP-treated hNPCs.

The drug MPTP interferes with the complex I of the electron transport chain and generates the ROS. Recent studies found that MPTP exposure could cause decreased proliferation and differentiation of NPCs isolated from the mice SVZ, which is similar to cellular senescence phenotype of NPC from aged mice [15]. Therefore, the current study used MPTP to treat NPCs and successfully establish a human NPC aging model. The current study provides a novel investigation concerning the oxidative stress–induced premature senescence of hESC-derived NPCs. This aging model enables us to extrapolate the molecular mechanism of hNSC aging.

Cell senescence is characterized by irreversible cell cycle arrest. which is mainly regulated by p16/RB and p53/p21 pathways [16]. According to the data obtained from the findings of this study, MPTP treatment induced upregulated expression of p21 and p53, which is associated with decreased proliferation. The expression level of SOD2 was downregulated after MPTP treatment. SOD2 is an enzyme that transforms toxic superoxide, a by-product of the mitochondrial electron transport chain, into hydrogen peroxide and diatomic oxygen. It has been shown that the overexpression of SOD2 increases lifespan by 20% [17]. Some data showed SOD2 deficiency could cause cellular senescence and aging phenotypes of skin through mitochondrial oxidative damage [18].

Autophagy is an important homeostatic cellular recycling mechanism responsible for degrading injured or dysfunctional cellular organelles in all living cells, and dysfunctional autophagy has also been observed in age-related diseases [19]. The results of this study showed that the expression of several key autophagy genes, such as Beclin 1, Atg5, Atg7, and Atg12, was downregulated after MPTP treatment. Recent studies have revealed that Atg5 and Beclin 1 are downregulated during normal human brain aging, and overexpression of Atg5 extends the median lifespan of mice by 17.2% [20].

Rapamycin is a pharmacological inhibitor of mTOR, which can increase lifespan in old mice. Previous studies showed that the aging phenotype of hematopoietic stem cells in old mice can be rejuvenated by rapamycin [21]. The current study found that rapamycin administration decreased the SA-β-gal expression and ROS level, increased the hNPC proliferation, and caused the downregulation of p53 and p21 and upregulation of SOD2 at the molecular level. Furthermore, our results showed that suppression of autophagy-related gene Beclin 1 attenuated rapamycin-induced rejuvenation of MPTP-induced human neural precursor cell senescence, indicating that autophagy function is involved in anti-senescence effect of rapamycin. These results demonstrated that autophagy induction can reverse the aging-associated phenotype of MPTP-treated hNPCs.

In conclusion, this study displayed a novel finding that hNSCs undergo a premature senescence induced by MPTP treatment, and this process can be reversed by stimulating autophagy through rapamycin administration. Uncovering the mechanisms of hNPC aging process improves the understanding of hNSC behavior and reveals tempting approaches for rejuvenating the aging of hNSCs.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the Ministry of Science and Technology of China (2011CB966200); the National Natural Science Foundation of China (31471029, 31301063, 81401053, 81461138037, 81472141); Public Health Bureau of Tianjin, 13KG127; National program for support of Top-notch young professionals (J.X.); Research Grant from Nantong University (13ZY005), Jiangsu Nature College Foundation (15KJB180014).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.174.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.174.

References

[1] B. Winner, Z. Kohl, F.H. Gage, Neurodegenerative disease and adult neurogenesis, Eur. J. Neurosci. 33 (2011) 1139–1151.
[2] E. Enwere, T. Shingo, C. Gregg, H. Fujikawa, S. Ohta, S. Weiss, Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory

neurogenesis, and deficits in fine olfactory discrimination, J. Neurosci. 24 (2004) 8354–8365.
[3] G.L. Ming, H. Song, Adult neurogenesis in the mammalian brain: significant answers and significant questions, Neuron 70 (2011) 687–702.
[4] R. Patani, P.A. Lewis, D. Trabzuni, C.A. Puddifoot, D.J. Wyllie, R. Walker, C. Smith, G.E. Hardingham, M. Weale, J. Hardy, S. Chandran, M. Ryten, Investigating the utility of human embryonic stem cell-derived neurons to model ageing and neurodegenerative disease using whole-genome gene expression and splicing analysis, J. Neurochem. 122 (2012) 738–751.
[5] F. L'Episcopo, C. Tirolo, N. Testa, S. Caniglia, M.C. Morale, M. Deleidi, M.F. Serapide, S. Pluchino, B. Marchetti, Plasticity of subventricular zone neuroprogenitors in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease involves cross talk between inflammatory and Wnt/beta-catenin signaling pathways: functional consequences for neuroprotection and repair, J. Neurosci. 32 (2012) 2062–2085.
[6] L.J. Chen, Y.Q. Gao, X.J. Li, D.H. Shen, F.Y. Sun, Melatonin protects against MPTP/MPP+ -induced mitochondrial DNA oxidative damage in vivo and in vitro, J. Pineal Res. 39 (2005) 34–42.
[7] F. L'Episcopo, C. Tirolo, N. Testa, S. Caniglia, M.C. Morale, M.F. Serapide, S. Pluchino, B. Marchetti, Wnt/beta-catenin signaling is required to rescue midbrain dopaminergic progenitors and promote neurorepair in ageing mouse model of Parkinson's disease, Stem Cells 32 (2014) 2147–2163.
[8] D.C. Rubinsztein, G. Marino, G. Kroemer, Autophagy and aging, Cell 146 (2011) 682–695.
[9] D.R. Green, L. Galluzzi, G. Kroemer, Mitochondria and the autophagy-inflammation-cell death axis in organismal aging, Science 333 (2011) 1109–1112.
[10] M.M. Lipinski, B. Zheng, T. Lu, Z. Yan, B.F. Py, A. Ng, R.J. Xavier, C. Li, B.A. Yankner, C.R. Scherzer, J. Yuan, Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 14164–14169.
[11] F. Madeo, N. Tavernarakis, G. Kroemer, Can autophagy promote longevity? Nat. Cell. Biol. 12 (2010) 842–846.
[12] D.E. Harrison, R. Strong, Z.D. Sharp, J.F. Nelson, C.M. Astle, K. Flurkey, N.L. Nadon, J.E. Wilkinson, K. Frenkel, C.S. Carter, M. Pahor, M.A. Javors, E. Fernandez, R.A. Miller, Rapamycin fed late in life extends lifespan in genetically heterogeneous mice, Nature 460 (2009) 392–395.
[13] S.C. Zhang, M. Wernig, I.D. Duncan, O. Brustle, J.A. Thomson, In vitro differentiation of transplantable neural precursors from human embryonic stem cells, Nat. Biotechnol. 19 (2001) 1129–1133.
[14] R.S. Balaban, S. Nemoto, T. Finkel, Mitochondria, oxidants, and aging, Cell 120 (2005) 483–495.
[15] F. L'Episcopo, C. Tirolo, N. Testa, S. Caniglia, M.C. Morale, F. Impagnatiello, S. Pluchino, B. Marchetti, Aging-induced Nrf2-ARE pathway disruption in the subventricular zone drives neurogenic impairment in parkinsonian mice via PI3K-Wnt/beta-catenin dysregulation, J. Neurosci. 33 (2013) 1462–1485.
[16] P. Kahlem, B. Dorken, C.A. Schmitt, Cellular senescence in cancer treatment: friend or foe? J. Clin. Invest 113 (2004) 169–174.
[17] C. Curtis, G.N. Landis, D. Folk, N.B. Wehr, N. Hoe, M. Waskar, D. Abdueva, D. Skvortsov, D. Ford, A. Luu, A. Badrinath, R.L. Levine, T.J. Bradley, S. Tavare, J. Tower, Transcriptional profiling of MnSOD-mediated lifespan extension in Drosophila reveals a species-general network of aging and metabolic genes, Genome Biol. 8 (2007) R262.
[18] M.C. Velarde, J.M. Flynn, N.U. Day, S. Melov, J. Campisi, Mitochondrial oxidative stress caused by Sod2 deficiency promotes cellular senescence and aging phenotypes in the skin, Aging (Albany NY) 4 (2012) 3–12.
[19] S. Giordano, V. Darley-Usmar, J. Zhang, Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease, Redox Biol. 2 (2014) 82–90.
[20] J.O. Pyo, S.M. Yoo, H.H. Ahn, J. Nah, S.H. Hong, T.I. Kam, S. Jung, Y.K. Jung, Overexpression of Atg5 in mice activates autophagy and extends lifespan, Nat. Commun. 4 (2013) 2300.
[21] C. Chen, Y. Liu, Y. Liu, P. Zheng, mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells, Sci. Signal 2 (2009) ra75.