Biochemical and Biophysical Research Communications 460 (2015) 397–403

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Roles of PTEN-induced putative kinase 1 and dynamin-related protein 1 in transient global ischemia-induced hippocampal neuronal injury

Shang-Der Chen [a, b, *], Tsu-Kung Lin [a], Ding-I. Yang [c], Su-Ying Lee [a], Fu-Zen Shaw [d], Chia-Wei Liou [a], Yao-Chung Chuang [a, b, *]

[a] Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taiwan
[b] Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taiwan
[c] Institute of Brain Science and Brain Research Center, National Yang-Ming University, Taipei, Taiwan
[d] Department of Psychology, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 28 January 2015
Available online 17 March 2015

Keywords:
Transient global ischemia
Hippocampus
PINK1
Drp1

ABSTRACT

Recent studies showed that increased mitochondrial fission is an early event of cell death during cerebral ischemia and dynamin-related protein 1 (Drp1) plays an important role in mitochondrial fission, which may be regulated by PTEN-induced putative kinase 1 (PINK1), a mitochondrial serine/threonine-protein kinase thought to protect cells from stress-induced mitochondrial dysfunction and regulate mitochondrial fission. However, the roles of PINK1 and Drp1 in hippocampal injury caused by transient global ischemia (TGI) remain unknown. We therefore tested the hypothesis that TGI may induce PINK1 causing downregulation of Drp1 phosphorylation to enhance hippocampal neuronal survival, thus functioning as an endogenous neuroprotective mechanism. We found progressively increased PINK1 expression in the hippocampal CA1 subfield1-48 h following TGI, reaching the maximal level at 4 h. Despite lack of changes in the expression level of total Drp1 and phosphor-Drp1 at Ser637, TGI induced a time-dependent increase of Drp1 phosphorlation at Ser616 that peaked after 24 h. Notably, PINK1-siRNA increased p-Drp1(Ser616) protein level in hippocampal CA1 subfield 24 h after TGI. The PINK1 siRNA also aggravated the TGI-induced oxidative DNA damage with an increased 8-hydroxy-deoxyguanosine (8-OHdG) content in hippocampal CA1 subfield. Furthermore, PINK1 siRNA also augmented TGI-induced apoptosis as evidenced by the increased numbers of TUNEL-positive staining and enhanced DNA fragmentation. These findings indicated that PINK1 is an endogenous protective mediator vital for neuronal survival under ischemic insult through regulating Drp1 phosphorylation at Ser616.

1. Introduction

PTEN-induced putative kinase 1 (PINK1) is a mitochondrial serine/threonine-protein kinase encoded by the PINK1 gene [1,2]. Mutations in this gene cause one form of autosomal recessive early-onset Parkinson disease [1,2]. Several studies suggested that PINK1 provides protection against mitochondrial dysfunction [3,4]. Mitochondria are known as the powerhouses in the cells vital for cellular homeostasis and participate in energy exchange, regulation of calcium homeostasis, and apoptosis [5]. Mitochondrial oxidative damage is considered to be a major factor in a wide variety of human disorders, including neurodegenerative diseases, aging, and cancer [6]. Excessive reactive oxygen species (ROS) generation can induce the functional and structural damage of hippocampus and may play an important role in the pathophysiology of forebrain ischemia [7–9].

Recent evidence also suggests that PINK1 plays an important role in mitochondrial quality control via autophagy machinery, in collaboration with parkin (a cytosolic E3 ligase) [10]. In mammalian cultured cells, PINK1 knockdown results in phenotypes of fragmented mitochondria [11]. It was reported that wild-type PINK1 protects against rotenone-induced mitochondrial fragmentation whereas PINK1-deficient cells show lower mitochondrial connectivity. Expression of dynamin-related protein 1 (Drp1) exaggerates the phenotypes associated with PINK1 deficiency, whereas Drp1 RNAi rescues them [12]. It has been noticed in recent years that

* Corresponding authors. Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, No. 123 Ta-Pei Road, Niao-Sung, Kaohsiung, 833, Taiwan. Fax: +886 7 7317123x2293.
E-mail addresses: chensd@adm.cgmh.org.tw (S.-D. Chen), ycchuang@adm.cgmh.org.tw (Y.-C. Chuang).

http://dx.doi.org/10.1016/j.bbrc.2015.03.045
0006-291X/

mitochondria are dynamic organelles and maintain their shape or morphology via 2 opposing processes, fission and fusion [13,14]. Mitochondrial fission involves the constriction and cleavage of mitochondria by fission proteins, such as Drp1 and mitochondrial fission 1 protein. The fusion process, on the other hand, involves the lengthening of mitochondria by tethering and joining together 2 adjacent mitochondria. Mitofusin-1 and -2 are mainly responsible for outer membrane fusion, while Opa1 is thought to mediate inner membrane fusion [13,14]. It was reported that mitochondrial fission occurs at 3 h after the onset of reperfusion, long before neuronal loss in focal cerebral ischemia model in mice, suggesting that fission is an upstream and early event in neuronal cell death [15]. In a recent study using oxygen-glucose deprivation (OGD) neuronal model, PINK1 was shown to significantly ameliorate OGD-induced cell death and energy disturbance by attenuating mitochondrial translocation of Drp1, which preserves mitochondrial function and inhibits OGD-induced mitochondrial fission [16]. These studies denote the potential role of PINK1/Drp1 in cerebral ischemia.

Given the facts that PINK1 seems to possess a pivotal role in regulating mitochondria dynamics and ROS formation, it is surprising that no *in vivo* study has been conducted to reveal the roles of PINK1 in cerebral ischemia. As Drp1 is emerged as a crucial player in ischemic paradigm [17–19], PINK1, an upstream kinase to modulate Drp1 expression, may also play a decisive role in cerebral ischemia. Given the intimate links among PINK1, Drp1, mitochondrial dynamics, and ROS in other neurodegenerative diseases such as Parkinson disease or Alzheimer's disease [20], delineation of this signaling pathway in cerebral ischemia should be rewarding in term of the mechanism research and the potential clinical application. In the present study, we therefore tested the hypothesis that PINK1 signaling pathway plays a protective effect in hippocampal CA1 subfield in TGI/reperfusion through down-regulation of Drp1 phosphorylation.

2. Materials and methods

2.1. Animals and general preparations

The experimental procedures used in this study conformed to the guidelines of our institutional committee for animal uses and research. All efforts were made to minimize animal suffering and to reduce the number of animals sacrificed. Adult male Sprague–Dawley rats (250–325 g) were purchased from the Experimental Animal Center, National Science Council, and BioLASCO, Taipei, Taiwan. They were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited animal facility under temperature control (24–25 °C) and 12-h light–dark cycle. An experimental model of TGI was performed as previously reported [8,9]. Briefly, the animals were subjected to a 10-min period of forebrain global ischemia by clamping both common carotid arteries and lowering blood pressure to 35–40 mm-Hg by withdrawing blood from a femoral arterial catheter; blood pressure were restored by infusing back the withdrawn blood afterwards. During anesthesia the core temperature was monitored and maintained at 37 ± 0.5 °C. The femoral artery were exposed and catheterized with a PE-50 catheter to allow continuous recording of the arterial blood pressure and to keep blood pressure in the designed range. After regaining consciousness, the animals were maintained in an air-conditioned room at 25 °C.

2.2. siRNA administration

All siRNAs were injected into bilateral hippocampal CA1 subfield as previously described [9]. To inhibit PINK1 expression, we used pre-designed PINK1-siRNA from MISSION® siRNA, (Sigma–Aldrich Ltd, St. Louis, MO, USA). The sequences of oligodeoxynucleotides were as follows: sense, 5′GCUGCAAUGCCGCUGUGUA3′, antisense, 5′ UACACGCGGCAUUGCAGC3′. For negative control siRNA (NC), the sequences of oligodeoxynucleotides were as follows: 5′GAUCAUAC GUGCGAUCAGA3′, antisense, 5′UCUGAUCGCACGUAUGAUC3′. The final concentration of siRNA was 0.05 nM in a total volume of 400 nl for injection into each side of hippocampal CA1 subfield 24 h before TGI.

2.3. Collection of tissue samples from the hippocampus

At pre-determined time-intervals (1, 4, 24, or 48 h) after induction of TGI, rats were again anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused intracardially with 50 mL of warm (37.8 °C) saline that contained heparin (100 U/mL). The brains were rapidly removed under visual inspection and placed on a piece of gauze moistened with ice-cold 0.9% saline. We routinely collect tissues from bilateral hippocampal CA1 area and these samples were stored at −80 °C until use [8,9]. The concentration of total proteins extracted from tissue samples was determined by the BCA Protein Assay (Pierce, Rockford, IL).

2.4. Western blotting

Samples (20–40 μg of proteins) were resolved through 2% SDS/polyacrylamide gels and transferred to PDVF membranes. The membranes were incubated with a primary anti-PINK1(1:1000), Drp1(1:500), p-Drp1(Ser616) (1:1000), and p-Drp1(Ser637) (1:1000) antibody (Cell Signaling, Danvers, MA, USA), for 1–2 h at room temperature. The membranes were washed with TBST 3 times at 10-min intervals, incubated with the secondary antibody (1:5000; anti-rabbit, anti-mouse, or anti-goat IgG conjugated with alkaline phosphatase; Promega, Madison, WI) at room temperature for 1 h, and then washed three times each at 10 min intervals with TBST and two times each for 10 min with TBS (TBST without Tween 20). In control experiments, a monoclonal antibody against α-tublin (1:10,000; Santa Cruz Biotecnology) was used. The color reaction was developed by the Blot AP System according to the technical manual provided by Promega.

2.5. Quantification of oxidative damage to DNA

Oxidative stress to the hipocampal CA1 subfield was evaluated by quantifying the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a marker for the oxidized DNA. The hippocampal CA1 were carefully isolated and total DNA purified (DNeasy Blood & Tissue kit; Qiagen, Valencia, CA) according to the manufacturer's instruction. The 8-OHdG (in ng/ml) were determined using a DNA oxidation kit (Highly Sensitive 8-OHdG Check ELISA; Japan Institute for the Control of Aging, Fukuroi, Japan) according to the manufacturer's protocol. Sample concentration from hippocampal CA1 subfield was calculated from a standard curve and corrected for DNA concentration.

2.6. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining

As a histochemical marker for apoptotic cell death, animals were processed for TUNEL staining 48 h after the onset of reperfusion following a 10-min episode of TGI. In brief, the hippocampus was removed and fixed in 30% sucrose in 10% formaldehyde-saline solution for ≥72 h. Six micrometer paraffin-embedded sections of the hippocampus were processed for TUNEL staining using an *in situ* apoptosis detection kit (ApopTag, Intergen Company, Purchase, NY,

USA). The total numbers of TUNEL-positive cells in each section were counted using an Olympus AX70 microscope and expressed as the TUNEL indices.

2.7. Qualitative and quantitative analysis of DNA fragmentation

Tissue samples from the hippocampal CA1 subfield were subjected to qualitative and quantitative analysis of DNA fragmentation as reported previously [9]. After extraction of total DNA from the hippocampal tissues, nucleosomal DNA ladders were amplified by a PCR kit for DNA ladder assay (Maxim Biotech, San Francisco, CA, USA) to enhance the detection sensitivity. Samples were separated by electrophoresis on 1% agarose gels. To quantify apoptosis-related DNA fragmentation, a cell death enzyme-linked immunosorbent assay (ELISA; Roche Molecular Biochemicals, Mannheim, Germany) was used to quantify the level of histone-associated DNA fragments in the cytoplasm [9]. The amount of nucleosomes in the cytoplasm was quantitatively according to the manufacturer's instruction (Anthros Labtec, Salzburg, Austria).

2.8. Statistical analysis

The continuous variables were expressed as mean ± SEM. One way analysis of variance followed by the Scheffé multiple range tests for post hoc assessment of individual means were used to compare the group mean differences. A p value less than 0.05 will be considered significant.

3. Results

3.1. Temporal changes of PINK1 and Drp1 expressions in the hippocampal CA1 subfield after TGI

Our first series of experiments examined whether PINK1 was induced by TGI in the hippocampal CA1 subfield. Western blot analysis revealed a significant increase of PINK1 expression in the total protein extracted from the hippocampal CA1 subfield 1–48 h after TGI, reaching the maximal level at 4 h (Fig. 1A). We then examined whether the expression of Drp1 may be affected by TGI in the hippocampal CA1 subfield. Results shown in Fig. 1B indicated that TGI with reperfusion up to 48 h failed to affect the expression level of total Drp1. Therefore, we investigated the potential effects of ischemia/reperfusion on the phosphorylation of Drp1. The division of the mitochondria is controlled in part by the phosphorylation of Drp1 at Ser616 by Cdk1/cyclin B and at Ser637 by protein kinase A (PKA). When phosphorylated at Ser616, Drp1 stimulates mitochondrial fission. Conversely, fission is inhibited when Drp1 is phosphorylated at Ser637 [21,22]. Results indicated that TGI

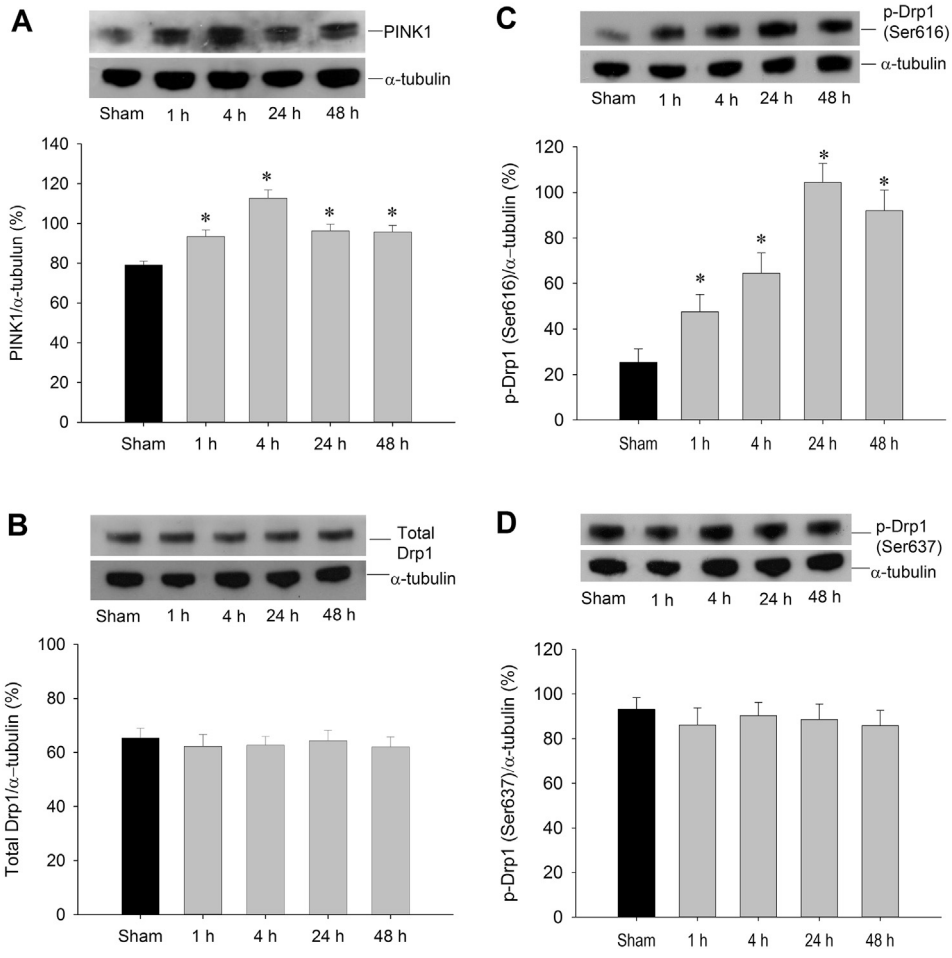

Fig. 1. Transient induction of PINK1 and p-Drp1(Ser616) by TGI/reperfusion. The rats were subjected to 10-min TGI followed by reperfusion for indicated times. Sham-operated animals served as negative controls. Hippocampal CA1 samples were then collected for western blotting to detect the expression of PINK1 (A), total Drp1 (B), p-Drp1(Ser616) (C), and p-Drp1(Ser637) (D). The same blots were also probed with α-tubulin antibody to serve as an internal reference control for equal loading of proteins in each lane. Values are mean ± SEM from representative blots and quantitative analyses from 5 to 7 animals in each experimental group are shown. *P < 0.05 versus sham control group in the Scheff'e multiple-range test.

induced a progressive augmentation of Drp1 phosphorylation at Ser616 in rat hippocampal CA1 regions that was peaked at 24 h after TGI (Fig. 1C); however, Drp1 phosphorylation at Ser637 (Fig. 1D) was not affected by TGI during the 48 h following the onset of reperfusion. Thus, TGI/reperfusion selectively induces phosphorylation of Drp1 at Ser616 in the CA1 subfield of the hippocampus in rats.

3.2. PINK1-siRNA silences PINK1 expression and augments p-Drp1(Ser616) expression in the hippocampal CA1 subfield after TGI

To further clarify the pivotal roles of PINK1 in this ischemic paradigm of the brain, we employed molecular approach by using PINK1-siRNA to elucidate the conception of underlying mechanisms. PINK1-siRNA successfully downregulated PINK1 expression in hippocampal CA1 subfield after TGI (Fig. 2A), resulting in approximately 60% suppression. Because our results shown in Fig. 1 indicated that TGI-induced PINK1 expression reached the peak level at 4 h, which preceded the maximal expression level of p-Drp1(Ser616) at 24 h. We therefore tested whether suppression of PINK1 may affect p-Drp1(Ser616) in ischemia/reperfusion. Results indicated that the PINK1 siRNA further augmented the extents of Drp1 phosphorylation at Ser616 that was induced by 10-min TGI with 24-h reperfusion (Fig. 2B), suggesting that TGI-induced PINK1 contributed to the suppression of Drp1 phosphorylation at Ser616.

3.3. Injection of PINK1-siRNA augments oxidative stress and increases neuronal injury in the hippocampal CA1 subfield after TGI

To further clarify the pivotal role of PINK1 in this ischemic paradigm of the brain, we investigated the effects of PINK1-siRNA over TGI-dependent oxidative stress and apoptosis. We found that pretreatment with PINK1-siRNA significantly augmented the extent of DNA oxidation, as evidenced by increased 8-OH-dG contents, in the hippocampal CA1 subfield 4 h after onset of reperfusion (Fig. 3A). Furthermore, suppression of PINK1 by siRNA also enhanced the extents of hippocampal neuronal apoptosis based on TUNEL staining (Fig. 3B), agarose gel electrophoresis to detect the extents of DNA fragmentation (Fig. 3C), and sandwich DNA fragmentation ELISA to quantitatively determine the amounts of oligonucleosomes in hippocampal tissue homogenates (Fig. 3D). These findings together revealed that inhibition of PINK1 expression may increase oxidative neuronal damage and apoptosis-related DNA fragmentation in the hippocampal CA1 subfield after TGI.

4. Discussion

The results demonstrated that TGI increased PINK1 and p-Drp1(Ser616) expression in the hippocampal CA1 subfield after ischemia/reperfusion. Pretreatment of PINK1-siRNA decreased

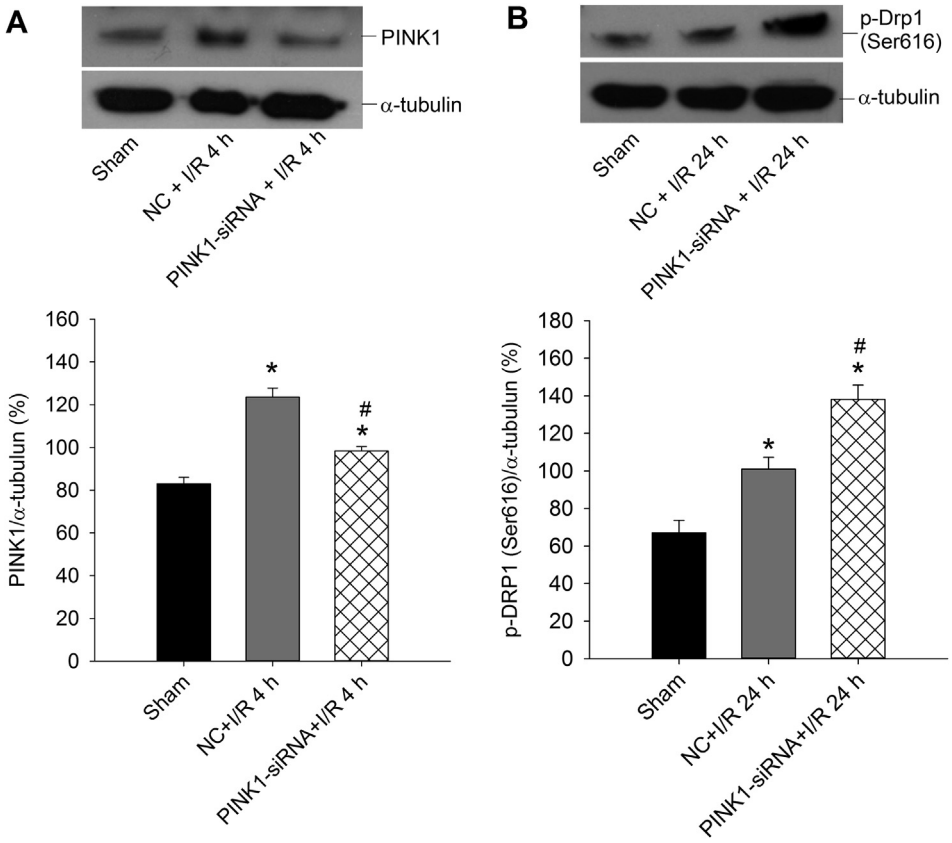

Fig. 2. PINK1-siRNA decreases PINK1 expression and increases p-Drp1(Ser616) expression in hippocampal CA1 subfield after TGI/reperfusion. (A). Rats were micro-injected into bilateral CA1 subfields with control siRNA or PINK1-siRNA 24 h before TGI. Total proteins of hippocampal CA1 subfield were collected from the sham-operated controls without siRNA microinjection (*Sham*), the rats injected with negative control siRNA followed by 10-min TGI and 4-h reperfusion (*NC+I/R 4 h*), or the rats injected with PINK1-siRNA followed by 10-min TGI and 4-h reperfusion (*PINK1-siRNA+ I/R 4 h*) before detection of PINK1 expression. (B) for the experimental condition was the same as in (A) except reperfusion time was 24 h and detection of p-Drp1(ser616) was conducted. In both (A) and (B), the same blots were also probed with an α-tubulin antibody to serve as an internal reference control for equal loading of proteins in each lane. Values are mean ± SEM from four to six animals per experimental group. *P < 0.05, vs. sham-control group; #P < 0.05 vs. control siRNA + TGI in the Scheff'e multiple-range test.

PINK1 expression while simultaneously augmented p-Drp1(Ser616) expression that was accompanied with heightened DNA oxidation and increased neuronal damage in the hippocampal CA1 subfield. These results suggested the protective role of PINK1 in TGI-induced neuronal injury in the hippocampal CA1 subfield, at least in part through regulating mitochondrial fission protein p-Drp1(Ser616) expression to protect neuronal cell under ischemic insult.

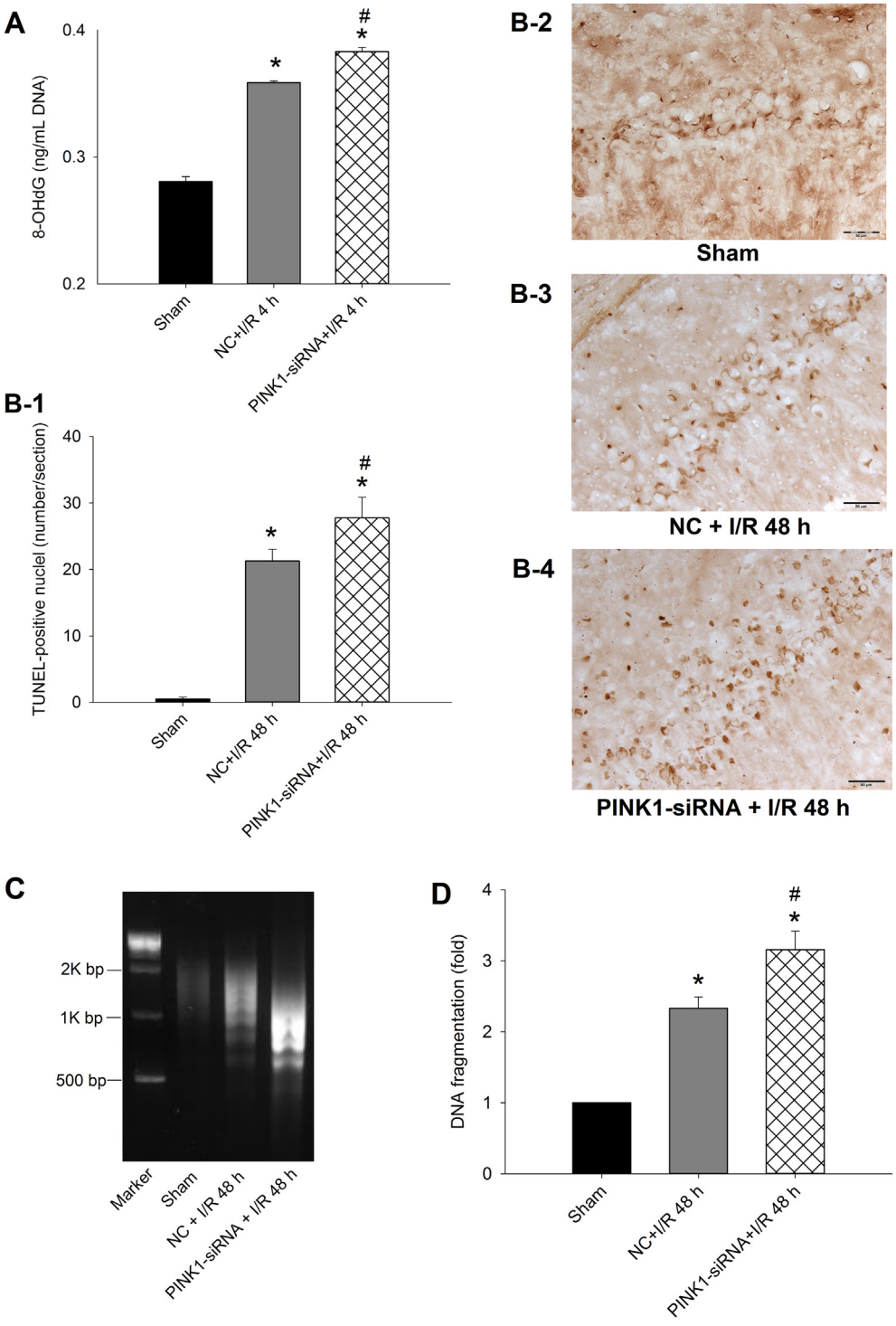

Fig. 3. PINK1-siRNA augments oxidative stress and apoptosis-related neuronal damage in hippocampal CA1 subfield after TGI. Rats were microinjected with negative control siRNA (*NC + I/R 4 h*) or the PINK1-siRNA (*PINK1-siRNA + I/R 4 h*) into the CA1 subfield 24 h before 10-min TGI with reperfusion for 4 h. The sham-operated controls without siRNA microinjection served as negative controls (*Sham*). The genomic DNA were isolated from collected hippocampal CA1 subfield to determine the amounts of 8-OHdG using a DNA oxidation kit (A). (B, C, D) The experimental condition was the same as described in (A) except that reperfusion time was 48 h. Hippocampal slices were subjected to TUNEL staining to determine the extents of apoptosis, as shown in (B). The genomic DNA was subjected to PCR assay to reveal apoptosis-related DNA fragmentation by agarose gel electrophoresis, as shown in (C). The tissue homogenates of hippocampal CA1 subfields were subjected to sandwich ELISA to assay the level of histone-associated DNA fragments in the cytoplasm as a quantitative measure for the DNA fragmentation, as shown in (D). Values in (A), (B-1), and (D) are mean ± SEM from 5 to 7 animals in each experimental group. *$P < 0.05$ vs. sham-control group and $^{\#}P < 0.05$ vs. negative control siRNA +I/R in the Scheff'e multiple-range test.

Recent studies showed that PINK1 is directly involved in regulating mitochondrial morphology [23]. Mitochondrial dysfunction can cause excessive oxidative stress and plays a crucial role in ischemic condition [7–9], whereas PINK1 possess a protective role against mitochondrial dysfunction [3,4]. However, studies of PINK1 in ischemia are limited [24–27]. In a recent study, loss of PINK1 increases the vulnerability of the heart to ischemia-reperfusion injury. This may contribute, in part, to increased mitochondrial dysfunction [24]. In studies of myocardial infarction, loss of FoxO1 and FoxO3 in cardiomyocytes results in a significant increase in infarct area with decreased expression of PINK1 [25]; resveratrol triggers an anti-aging pathway in the mitochondria that involves Sirt3-Foxo3a-PINK1-PARKIN-mitochondrial fusion-fission-mitophagy [27]. These studies denote the potential protective effects of PINK1 under heart ischemia. In a rabbit spinal cord ischemia model, induction of PINK1 proteins in the motor neurons at the early stage of reperfusion is related to excessive oxidative stress [26]. While the exact function of PINK1 in cerebral ischemia is yet to be elucidated, evidence of the protective role of PINK1 in various ischemic condition [24–27] suggests that PINK1 possesses a protective role under cerebral ischemia. In this study, we showed that the PINK1 expression is activated in hippocampal CA1 subfield under TGI, which implies a potential role PINK1 in this ischemic condition (Fig. 1A). In a recent *in vitro* study using OGD-induced neuronal death, which included neuron from the cortex and hippocampus, the authors showed that PINK1 significantly ameliorated OGD-induced cell death and energy disturbance [16]. Based on this *in vitro* model of cerebral hypoxia/ischemia, these results argue a potentially pivotal role of PINK1 in cerebral ischemia.

In a recent study, it has been reported that a progressive activation of Drp1 in its phosphorylation form is related to apoptotic process in neural cells of the peri-infarct regions after focal cerebral ischemia [28]. Drp1 is a large GTPase that cycles between the cytosol and outer mitochondria membrane. Several notable post-translational modifications of Drp1 influence its function, which include phosphorylation, S-nitrosylation, ubiquitination, and sumoylation [29]. Among them, phosphorylation is an important part of regulation in term of mitochondrial dynamics [29] and multiple phosphorylation sites of Drp1 have been characterized for their functional importance [21]. Drp1 Ser637 was identified as a phosphorylation site by PKA that can lead to elongated mitochondria [30,31]. In contrast, phosphorylation at Ser616 of Drp1 (equivalent to Ser585 of rat Drp1) by CDK1/Cyclin B induces mitochondrial fission [21,32]. Phosphorylation at Ser637 by PKA induces elongated mitochondria that may protect cells against pro-apoptotic stimuli. On the contrary, phosphorylation at Ser616 leads to an apoptotic cascade [21,31,32]. In this study, we showed a significant change of p-Drp1(Ser616), but not Ser637, in the hippocampal CA1 subfield after TGI, which may denote an increase of mitochondrial fission in hippocampal CA1 subfield after TGI (Fig. 1C).

It was reported recently that after exposure of cultured neurons to oxidative and metabolic stress, mitochondria exhibited fragmentation and increased levels of the fission protein Drp1 [18]. In human neuroblastoma cell line SH-SY5Y, over-expression of PINK1 reversed OGD-induced increases in mitochondrial fragmentation and suppressed the translocation of Drp1 from the cytosol to the mitochondria, while knockdown of PINK1 significantly increased the severity of OGD-induced neuronal damage [16]. These results disclosed the opposing effect between PINK1 and Drp1 over mitochondrial dynamic under stressful condition. Our results showed that PINK1-siRNA successfully decreased PINK1 expression (Fig. 2A) and, importantly, increased p-Drp1(Ser616) expression, suggesting a regulatory role of PINK1 over Drp1 in this ischemic paradigm (Fig. 2B). These results are in accordance with the augmentation of oxidative DNA damage and more neuronal apoptosis over hippocampal CA1 subfield under TGI (Fig. 3).

In conclusion, this study showed that PINK1 may play as an endogenous protective mechanism in cerebral ischemic condition which affects p-Drp1(Ser616) expression, modulate oxidative status, and influence neuronal damage. Any measurement to enhance PINK1 expression in cerebral ischemia should have clinical prospective in counteracting ROS- and Drp1-related neuronal damage.

Conflict of interest

None.

Acknowledgments

This work was supported by research grants NSC 99-2314-B-182A-069- and 101-2314-B-182-081-MY2 to S.D. Chen from the National Science Council, Taiwan.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.045.

References

[1] M. Unoki, Y. Nakamura, Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway, Oncogene 20 (2001) 4457–4465.
[2] E.M. Valente, S. Salvi, T. Ialongo, R. Marongiu, A.E. Elia, V. Caputo, L. Romito, A. Albanese, B. Dallapiccola, A.R. Bentivoglio, PINK1 mutations are associated with sporadic early-onset parkinsonism, Ann. Neurol. 56 (2004) 336–341.
[3] J.W. Pridgeon, J.A. Olzmann, L.S. Chin, L. Li, PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1, PLoS Biol. 5 (2007) e172.
[4] H. Plun-Favreau, K. Klupsch, N. Moisoi, S. Gandhi, S. Kjaer, D. Frith, K. Harvey, E. Deas, R.J. Harvey, N. McDonald, N.W. Wood, L.M. Martins, J. Downward, The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1, Nat. Cell. Biol. 9 (2007) 1243–1252.
[5] K. Niizuma, H. Endo, P.H. Chan, Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival, J. Neurochem. 109 (Suppl. 1) (2009) 133–138.
[6] J.V. Leonard, A.H. Schapira, Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects, Lancet 355 (2000) 389–394.
[7] T. Hayashi, A. Saito, S. Okuno, M. Ferrand-Drake, R.L. Dodd, T. Nishi, C.M. Maier, H. Kinouchi, P.H. Chan, Oxidative damage to the endoplasmic reticulum is implicated in ischemic neuronal cell death, J. Cereb. Blood Flow. Metab. 23 (2003) 1117–1128.
[8] S.D. Chen, H.Y. Wu, D.I. Yang, S.Y. Lee, F.Z. Shaw, T.K. Lin, C.W. Liou, Y.C. Chuang, Effects of rosiglitazone on global ischemia-induced hippocampal injury and expression of mitochondrial uncoupling protein 2, Biochem. Biophys. Res. Commun. 351 (2006) 198–203.
[9] S.D. Chen, T.K. Lin, D.I. Yang, S.Y. Lee, F.Z. Shaw, C.W. Liou, Y.C. Chuang, Protective effects of peroxisome proliferator-activated receptors gamma coactivator-1alpha against neuronal cell death in the hippocampal CA1 subfield after transient global ischemia, J. Neurosci. Res. 88 (2010) 605–613.
[10] S. Kawajiri, S. Saiki, S. Sato, N. Hattori, Genetic mutations and functions of PINK1, Trends Pharmacol. Sci. 32 (2011) 573–580.
[11] N. Exner, B. Treske, D. Paquet, K. Holmstrom, C. Schiesling, S. Gispert, I. Carballo-Carbajal, D. Berg, H.H. Hoepken, T. Gasser, R. Kruger, K.F. Winklhofer, F. Vogel, A.S. Reichert, G. Auburger, P.J. Kahle, B. Schmid, C. Haass, Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin, J. Neurosci. 27 (2007) 12413–12418.
[12] A. Sandebring, K.J. Thomas, A. Beilina, M. van der Brug, M.M. Cleland, R. Ahmad, D.W. Miller, I. Zambrano, R.F. Cowburn, H. Behbahani, A. Cedazo-Minguez, M.R. Cookson, Mitochondrial alterations in PINK1 deficient cells are influenced by calcineurin-dependent dephosphorylation of dynamin-related protein 1, PLoS One 4 (2009) e5701.
[13] D.C. Chan, Mitochondrial fusion and fission in mammals, Annu Rev. Cell. Dev. Biol. 22 (2006) 79–99.
[14] B. Westermann, Molecular machinery of mitochondrial fusion and fission, J. Biol. Chem. 283 (2008) 13501–13505.
[15] M.J. Barsoum, H. Yuan, A.A. Gerencser, G. Liot, Y. Kushnareva, S. Graber, I. Kovacs, W.D. Lee, J. Waggoner, J. Cui, A.D. White, B. Bossy, J.C. Martinou, R.J. Youle, S.A. Lipton, M.H. Ellisman, G.A. Perkins, E. Bossy-Wetzel, Nitric

oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons, EMBO J. 25 (2006) 3900–3911.
[16] Y. Zhao, F. Chen, S. Chen, X. Liu, M. Cui, Q. Dong, The Parkinson's disease-associated gene PINK1 protects neurons from ischemic damage by decreasing mitochondrial translocation of the fission promoter Drp1, J. Neurochem. 127 (2013) 711–722.
[17] J.X. Wang, J.Q. Jiao, Q. Li, B. Long, K. Wang, J.P. Liu, Y.R. Li, P.F. Li, miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1, Nat. Med. 17 (2011) 71–78.
[18] D. Liu, D.L. Croteau, N. Souza-Pinto, M. Pitta, J. Tian, C. Wu, H. Jiang, K. Mustafa, G. Keijzers, V.A. Bohr, M.P. Mattson, Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions, J. Cereb. Blood Flow. Metab. 31 (2011) 680–692.
[19] S.B. Ong, S. Subrayan, S.Y. Lim, D.M. Yellon, S.M. Davidson, D.J. Hausenloy, Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury, Circulation 121 (2010) 2012–2022.
[20] M.H. Yan, X. Wang, X. Zhu, Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease, Free Radic. Biol. Med. 62 (2013) 90–101.
[21] A.B. Knott, G. Perkins, R. Schwarzenbacher, E. Bossy-Wetzel, Mitochondrial fragmentation in neurodegeneration, Nat. Rev. Neurosci. 9 (2008) 505–518.
[22] R.J. Giedt, C. Yang, J.L. Zweier, A. Matzavinos, B.R. Alevriadou, Mitochondrial fission in endothelial cells after simulated ischemia/reperfusion: role of nitric oxide and reactive oxygen species, Free Radic. Biol. Med. 52 (2012) 348–356.
[23] Y. Imai, B. Lu, Mitochondrial dynamics and mitophagy in Parkinson's disease: disordered cellular power plant becomes a big deal in a major movement disorder, Curr. Opin. Neurobiol. 21 (2011) 935–941.
[24] H.K. Siddall, D.M. Yellon, S.B. Ong, U.A. Mukherjee, N. Burke, A.R. Hall, P.R. Angelova, M.H. Ludtmann, E. Deas, S.M. Davidson, M.M. Mocanu, D.J. Hausenloy, Loss of PINK1 increases the heart's vulnerability to ischemia-reperfusion injury, PLoS One 8 (2013) e62400.
[25] A. Sengupta, J.D. Molkentin, J.H. Paik, R.A. DePinho, K.E. Yutzey, FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress, J. Biol. Chem. 286 (2011) 7468–7478.
[26] M. Sakurai, T. Kawamura, H. Nishimura, H. Suzuki, F. Tezuka, K. Abe, Induction of Parkinson disease-related proteins in motor neurons after transient spinal cord ischemia in rabbits, J. Cereb. Blood Flow. Metab. 29 (2009) 752–758.
[27] S. Das, G. Mitrovsky, H.R. Vasanthi, D.K. Das, Antiaging properties of a grape-derived antioxidant are regulated by mitochondrial balance of fusion and fission leading to mitophagy triggered by a signaling network of Sirt1-Sirt3-Foxo3-PINK1-PARKIN, Oxid. Med. Cell. Longev. 2014 (2014) 345105.
[28] W. Liu, F. Tian, T. Kurata, N. Morimoto, K. Abe, Dynamic changes of mitochondrial fusion and fission proteins after transient cerebral ischemia in mice, J. Neurosci. Res. 90 (2012) 1183–1189.
[29] T.J. Wilson, A.M. Slupe, S. Strack, Cell signaling and mitochondrial dynamics: implications for neuronal function and neurodegenerative disease, Neurobiol. Dis. 51 (2013) 13–26.
[30] C.R. Chang, C. Blackstone, Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology, J. Biol. Chem. 282 (2007) 21583–21587.
[31] G.M. Cereghetti, A. Stangherlin, O. Martins de Brito, C.R. Chang, C. Blackstone, P. Bernardi, L. Scorrano, Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria, Proc. Natl. Acad. Sci. U S A 105 (2008) 15803–15808.
[32] N. Taguchi, N. Ishihara, A. Jofuku, T. Oka, K. Mihara, Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission, J. Biol. Chem. 282 (2007) 11521–11529.