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Research Paper

Mutation of *hop-1* and *pink-1* attenuates vulnerability of neurotoxicity in *C. elegans*: the role of mitochondria-associated membrane proteins in Parkinsonism

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ABSTRACT

Mitochondrial dysfunction is considered as a critical mechanism in the pathogenesis of Parkinson's disease (PD). Increasing evidence supports the notion of mitochondria-associated membranes (MAMs) in mitochondrial dysfunction; yet little is known about the role of MAMs-related proteins in the pathogenesis of PD. Herein we exposed the nematode *Caenorhabditis elegans* to 0.5–10.0 μM rotenone (RO) or 0.2–1.6 mM paraquat (PQ) for 3 days. Our results showed that both RO and PQ induced similar Parkinsonism including motor deficits and dopaminergic degeneration. RO/PQ caused mitochondrial damages characterized by the increase of vacuole areas and autophagy vesicles, but the decrease of mitochondrial cristae. RO/PQ-impacted mitochondrial function was also demonstrated by the decrease of ATP level and mitochondrial membrane potential. Additionally, the attachment or surrounding of endoplasmic reticulum to the damaged mitochondria indicates ultrastructural alterations in MAMs. Using fluorescently labeled transgenic nematodes, we further found that the expression of *tomm-7* and genes of Complex I, II and III was reduced, whereas the expression of *pink-1* was increased in the exposed animals. To determine MAMs in toxicity toward PD, we investigated the mutants of *hop-1* and *pink-1*, encoding presenilin and PTEN-induced putative kinase 1 (PINK1) in mitochondria-associated membranes, respectively. Results demonstrated that the mutation of both *hop-1* and *pink-1* reduced the vulnerability of lethal, behavioral, and mitochondrial toxicity induced by RO/PQ. These findings suggest that presenilin and PINK1 play important roles in the RO/PQ-induced neurotoxicity through the mechanisms involved in mitochondria-associated membranes.

1. Introduction

Parkinson's disease (PD) is the common neurodegenerative disease characterized by the loss of dopaminergic neurons. Mutations in several genes have been proved to be linked to familial PD and genetics is expected to explain 5–10% of PD cases; however, about 90% of PD cases are sporadic (Pickrell and Youle, 2015; Yu et al., 2018). Until now, the etiology of sporadic PD remains poorly understood. The prevailing hypothesis is that the sporadic PD is most likely associated with environmental toxicants including pesticides and heavy metals (Bellou et al., 2016). Epidemiological studies have proved that pesticide exposure is associated with an increased risk of developing PD (Goldman, 2014). It is also supported by evidence from cell-based and animal studies (Li and Le, 2013; Bellou et al., 2016; Ko and Bezard, 2017). Rotenone and paraquat are the common pesticide or herbicide widely used. Previous studies have demonstrated that both rotenone and paraquat can kill dopaminergic neurons *in vitro* (Sala et al., 2016). Chronic administrations of paraquat or rotenone could cause key features of PD, including motor deficits and loss of dopaminergic neurons (Tanner et al., 2011). Although rotenone and paraquat were utilized for the PD model, the toxic mechanisms of neurodegeneration remain to be determined (Spivey, 2011).

Mitochondrial dysfunction has been proposed as one of the major contributors to the pathogenesis of PD (Schon and Przedborski, 2011; Esteves et al., 2014; Surmeier et al., 2017). Dysfunction of mitochondrial respiratory chain components was found in the brain, skeletal

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muscle, and platelets of sporadic PD patients (van der Merwe et al., 2014). PD-related genes including *parkin*, *PINK1*, *DJ-1*, and *LRRK2* are involved in mitochondrial regulation, and mutations of these genes are related to mitochondrial dysfunction (Thomas et al., 2011; Smith et al., 2015). Recent studies show that communication between mitochondria and the endoplasmic reticulum (ER) plays key roles in the regulation of various pathophysiological processes (Giorgi et al., 2015; Filadi et al., 2016). Mitochondria-associated membranes (MAMs) are specific regions of contact between mitochondria and the endoplasmic reticulum, which attract increasing attention about their function and physiological consequences (Rodríguez-Arribas et al., 2017). To date, there is a little known about the role of MAMs-related proteins in the toxicity associated with PD (Hattori et al., 2017).

Several proteins located in MAMs, including those encoded by PD-related genes and some ER proteins such as presenilin, are involved in mitochondrial regulation (Gelmetti et al., 2017). As one of the PD-related proteins, PTEN-induced putative kinase 1 (PINK1) is a mitochondrial serine/threonine-protein kinase and takes part in the protection of stress-induced mitochondrial dysfunction (Lazarou et al., 2013). PINK1 interacts with the proautophagic protein BECN1/Beclin1 that is relocalized at MAMs during the process of starvation-induced autophagy (Gelmetti et al., 2017). PINK1 causes the parkin protein to bind into depolarized mitochondria, in order to induce autophagy of those mitochondria (Narendra et al., 2010; Lazarou et al., 2013). On the other hand, presenilin is involved in the control of mitochondrial functions (Filadi et al., 2017), and enriched in MAMs. Presenilin possess a domain involved in different pathways related to ER and mitochondrial functions (van Vliet et al., 2014; Filadi et al., 2017). However, little is known about the potential roles of MAMs-related proteins, including both PINK1 and presenilin, in the mitochondrial toxicity associated with sporadic PD (Checler et al., 2017).

The nematode *Caenorhabditis elegans* is widely used as an important animal model with the properties of a short life cycle, transparent body, easy handling, well-defined anatomy and well-described genetic and molecular backgrounds (Xu et al., 2017). With only 302 neurons in its nervous system and the lineage and morphology of every neuron described comprehensively, *C. elegans* also acts as an excellent model organism for neurotoxicology study. *C. elegans* lacks a functional blood brain barrier; therefore, chemical neurotoxins can quickly diffuse into the nervous system. The nematode *C. elegans* shares wide homologous genome with mammals. For example, *pink-1* and *hop-1* in nematodes are the homologous genes of PINK1 and presenilin in mammals, respectively (Li and Greenwald, 1997; Sämann et al., 2009). Therefore, *C. elegans* has been used as an outstanding model for neurodegenerative diseases such as PD (Caito and Aschner, 2016).

Herein we exposed *C. elegans* to either rotenone or paraquat to induce behavioral and pathological characteristics associated with Parkinsonism. The morphological changes of mitochondria, endoplasmic reticulum and MAMs were examined by transmission electron microscopy. Using fluorescently-labeled transgenic nematodes, we also investigated the mitochondrial toxicity for its linkage with MAMs-related proteins. We next tested the vulnerability of lethal, behavioral and mitochondrial toxicity induced by rotenone/paraquat in the mutant of *hop-1* or *pink-1*. Our results indicate that the loss of PINK1 and presenilin functions impairs the toxicity of rotenone and paraquat, identifying the importance of both proteins in neurotoxicity. These experiments provide detailed *in vivo* evidence for MAMs mechanisms of environment-related Parkinsonism.

2. Materials and methods

2.1. Chemicals

Rotenone (RO) was purchased from Aladdin Industrial Corporation (Shanghai, China). Paraquat (Methyl viologen dichloride hydrate, PQ) was purchased from Sigma Aldrich Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals used in this study were of analytical grade.

2.2. C. elegans strains

All strains of *C. elegans* were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA) and maintained following standard protocols as previously described (Sulston and Brenner, 1974). Transgenic strains of *C. elegans* used in this study include the following: *BZ555* [*dat-1p::GFP*] (Jadiya et al., 2011); *PD4251* [*(pSAK2) myo-3p::GFP::LacZ::NLS + (pSAK4) myo-3p::mitochondrial GFP + dpy-20(+)*] (Cao et al., 2007); *CB7272* [[(pSAK2) *myo-3p::GFP::LacZ::NLS* + (pSAK4) *myo-3p::mitochondrial* GFP + dpy-20(+)] I. mIs12 [*myo-2p::GFP + pes-10p::GFP + F22B7.9p::GFP*] II. frIs7 [*nlp-29p::GFP + col-12p::DsRed]* IV. uIs69 [*pCFJ90(myo-2p::mCherry) + unc-119p::sid-1*] V] (Thompson et al., 2013); *DLM14* [*eft-3p::CERULEAN-VENUS::tomm-7 + unc-119(+)*] (Chapin et al., 2015); *LA62* [*hop-1mt*] (Lakowski et al., 2003); *BR4006* [*pink-1p::pink-1::GFP + myo-2p::mCherry* + herring sperm DNA] (Sämann et al., 2009) and *RB2547* [*pink-1(ok3538)*].

2.3. Nematode maintenance and exposure

Nematodes were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli OP50* at 20 °C (Sulston and Brenner, 1974). Adult nematodes were collected into tubes, and then dissolved a bleach-containing buffer (0.45 mM NaOH, 2% HClO) to release eggs. The eggs were collected and hatched on the plates with food, then synchronized for experiments (Xu et al., 2016).

Rotenone was initially dissolved in dimethyl sulfoxide (DMSO) and diluted with K-medium (32 mM KCl, 51 mM NaCl). PQ solutions were prepared in K-medium. The control group was K-medium. According to results of lethality assays, sublethal concentrations of RO (0.5, 1.0, 2.0, 4.0, 8.0, 10.0 μM) or PQ (0.2, 0.4, 0.6, 0.8, 1.2, 1.6 mM) were used for further research. Nematodes were exposed in the 24-well plates. Each well contained 30–50 age-synchronized nematodes and 45 μL *E. coli OP50* solutions for food. Each experimental group included four parallels. L2 stage nematodes were exposed for 3 days.

2.4. Lethality assays

Synchronized wild type or transgenic nematodes were exposed to RO or PQ for 6 days in 24-well plates. To prevent eggs from hatching, 3.3 μL of fluoro-29-deoxyuridine (150 mM) was added to each well. The numbers of dead nematodes were recorded every day. Dead nematodes were identified as unresponsive to a gentle prod of the body with a needle probe. Each experiment was run in quadruplicate. The median lethal concentrations (LC50) of RO and PQ were determined by linear regression analysis with Graphpad Prism (Xu et al., 2017).

2.5. Movement behavior

Body bending was utilized to evaluate locomotion behaviors of nematodes. After the exposure, nematodes were transferred to K-medium. After one minute of recovery, body bending was counted in 20s. Additionally, *C. elegans* was transferred to surface of fresh NGM after exposure experiments. Following 10 min of adaptation, videos of nematodes were recorded for 1 min using the Motic microscope and Images Advanced 3.2 software (Motic Electric Group, Xiamen, China). Locomotion videos were further analyzed by Wormlab software (MBF Bioscience, Hong Kong, China). The crawling tracks were calculated using the midpoint of the body of nematodes in terms of references (Donnelly et al., 2013). Crawling speeds were recorded in real-time using a bin width of 0.125 s.