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Activation of AMPK and inactivation of Akt result in suppression of mTOR-mediated S6K1 and 4E-BP1 pathways leading to neuronal cell death in *in vitro* models of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons. Dysregulation of mammalian target of rapamycin (mTOR) has been implicated in the pathogenesis of PD. However, the underlying mechanism is incompletely elucidated. Here, we show that PD mimetics (6-hydroxydopamine, N-methyl-4-phenylpyridine or rotenone) suppressed phosphorylation of mTOR, S6K1 and 4E-BP1, reduced cell viability, and activated caspase-3 and PARP in PC12 cells and primary neurons. Overexpression of wild-type mTOR or constitutively active S6K1, or downregulation of 4E-BP1 in PC12 cells partially prevented cell death in response to the PD toxins, revealing that mTOR-mediated S6K1 and 4E-BP1 pathways due to the PD toxins were inhibited, leading to neuronal cell death. Furthermore, we found that the inhibition of mTOR signaling contributing to neuronal cell death was attributed to suppression of Akt and activation of AMPK. This is supported by the findings that ectopic expression of constitutively active Akt or dominant negative AMPKα, or inhibition of AMPKα with compound C partially attenuated inhibition of phosphorylation of mTOR, S6K1 and 4E-BP1, activation of caspase-3, and neuronal cell death triggered by the PD toxins. The results indicate that PD stresses activate AMPK and inactivate Akt, causing neuronal cell death via inhibiting mTOR-mediated S6K1 and 4E-BP1 pathways. Our findings suggest that proper co-manipulation of AMPK/Akt/mTOR signaling may be a potential strategy for prevention and treatment of PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by progressive loss of central and peripheral neurons, and especially the substantia nigra pars compacta (SNc) dopaminergic neurons [1–3]. To understand the mechanism of neuronal cell death and develop neuroprotective therapies in PD, numerous studies have been carried out in postmortem brains as well as experimental cell and animal models of PD [3–7]. Although the precise etiology of PD is unknown, increasing evidence has pointed to the association of neuronal loss with apoptosis, which can be triggered by oxidative stress, impairment of mitochondrial respiration, and abnormal protein aggregation [4,5,8]. Especially, recent data have shown that dysregulation of mammalian target of rapamycin (mTOR) is implicated in the pathogenesis of PD [9,10]. However, the underlying mechanism is incompletely elucidated.

mTOR, a serine/threonine (Ser/Thr) protein kinase, is a central controller of cell proliferation, growth and survival [11,12]. mTOR functions at least as two complexes, mTORC1 and mTORC2, with distinct substrate specificities [12]. mTORC1 regulates phosphorylation of p70 S6 kinase 1

Abbreviations: 4E-BP1, eukaryotic initiation factor 4E binding protein 1; 6-OHDA, 6-hydroxydopamine; ACC, acetyl-CoA carboxylase; AD, Alzheimer's disease; AMPK, AMP-dependent/activated protein kinase; DAB, 3,3′-diaminobenzidine tetrachloride; DAPI, 4′,6-diamidino-2-phenylindole; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; HA, hemagglutinin; HD, Huntington's disease; MPP^+, 1-Methyl-4-phenylpyridin-1-ium; mTOR, mammalian target of rapamycin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PARP, poly ADP-ribose polymerase; PBS, phosphate buffered saline; PD, Parkinson's disease; PDK1/2, 3-Phosphoinositide-dependent protein kinase-1/2; PDL, poly-D-lysine; PI3K, phosphatidylinositol 3′-kinase; Akt (PKB), protein kinase B; S6K1, ribosomal p70 S6 kinase 1; Ser/Thr, serine/threonine.

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(S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) [11–13], whereas mTORC2 phosphorylates Akt on Ser473 [14]. In addition to phosphorylation at Ser473, Akt activity is also positively regulated by phosphorylation at Thr308, which is mediated by phosphoinositide-dependent kinase 1 (PDK1) and requires activation of phosphatidylinositol 3′-kinase (PI3K) signaling [11,12,14–17]. Activated PI3K catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PIP3). Increased PIP3 binds to the pleckstrin homology domain of Akt and, in combination with additional Ser/Thr phosphorylation of Akt by PDK1 and mTORC2, results in full activation of Akt. Subsequently, activated PI3K or Akt may positively regulate mTOR, leading to increased phosphorylation of S6K1 and 4E-BP1, the two best-characterized downstream effector molecules of mTOR [11–13]. Numerous studies have demonstrated that mTOR regulates differentiation and survival in neurons, and plays an important role in synaptic plasticity, learning and memory, and food uptake in adult brain [18,19]. mTOR activity is modified in various pathologic states of the nervous system, including brain tumors, tuberous sclerosis, cortical dysplasia and neurodegenerative disorders such as PD, Alzheimer's disease (AD), and Huntington's disease (HD) [19]. Active Akt, as a major regulator of neuronal cell survival [20], is negatively associated with dopaminergic neurodegeneration in PD [2,21]. Additionally, AMP-activated protein kinase (AMPK) is activated in response to oxidative stress [22], and also plays a critical role in neurodegenerative diseases [23,24]. Multiple studies have demonstrated that Akt can be inhibited by 6-OHDA or hydrogen peroxide (H_2O_2) in neuronal cells [2,9,21,25]. H_2O_2 induces neuronal apoptosis by activation of AMPK, leading to suppression of mTOR pathway [25]. This prompts us to study how both AMPK and Akt activities influence mTOR signaling pathway contributing to neuronal cell death in the context of PD.

Here, we show both activation of AMPK and inactivation of Akt in *in vitro* PD models, which contributes to suppression of mTOR-mediated S6K1 and 4E-BP1 pathways and induction of neuronal cell death. Our findings suggest that proper co-manipulation of AMPK/Akt/mTOR signaling may be a potential strategy for prevention and treatment of PD.

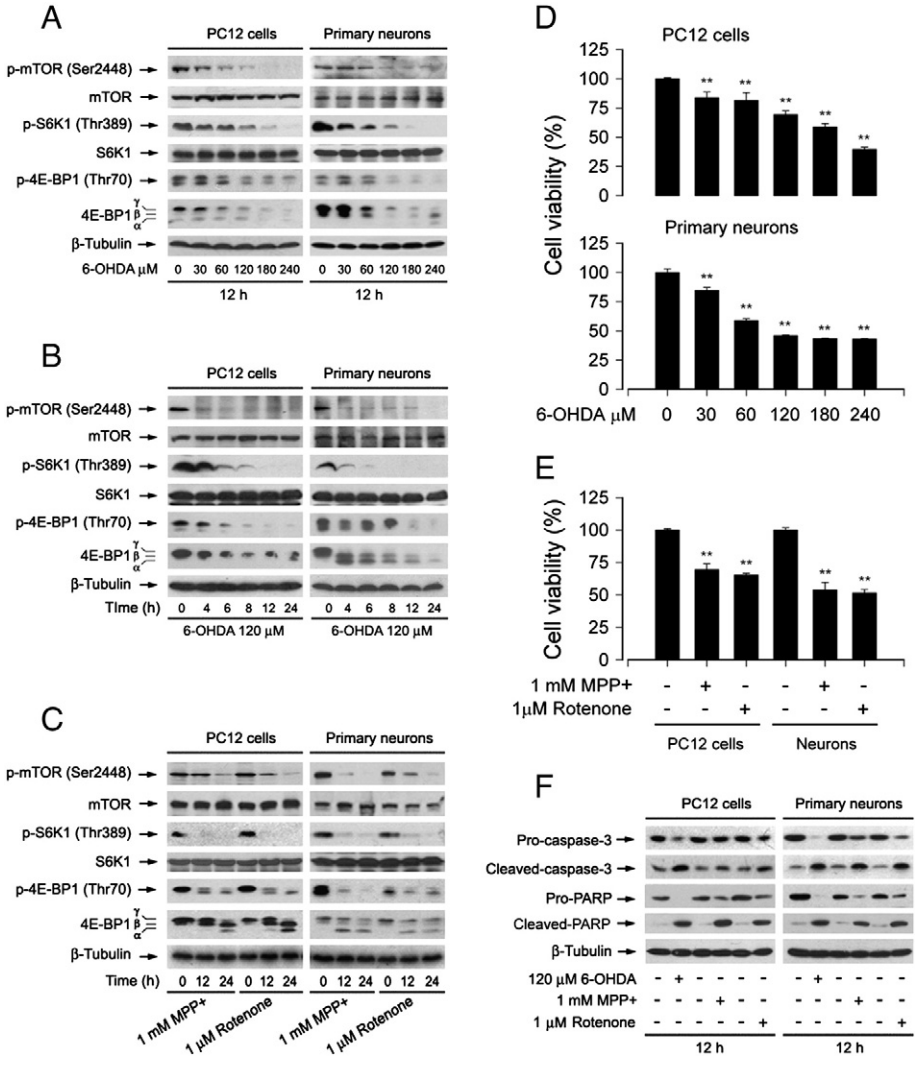

Fig. 1. Inhibition of mTOR signaling pathway is associated with neuronal cell death in cellular models of PD. PC12 cells and primary neurons were treated with 6-OHDA (0–240 μM or 120 μM), MPP^+ (1 mM) or rotenone (1 μM) for 12 and/or 24 h, as described in the Materials and methods section. Total cell lysates were subjected to Western blotting using indicated antibodies. The blots were probed for β-tubulin as a loading control. Similar results were observed in at least three independent experiments. Cell viability was evaluated using an MTT assay. (A and B) 6-OHDA (0–240 μM for 12 h or 120 μM for 0–24 h) obviously inhibited phosphorylation of mTOR, S6K1, and 4E-BP1 in a concentration- and time-dependent manner, and (C) treatment with MPP^+ (1 mM) or rotenone (1 μM) for 12 and 24 h also inhibited mTORC1 signaling in PC12 cells and primary neurons. (D) Treatment with 6-OHDA (0–240 μM) for 24 h concentration-dependently reduced cell viability, and (E) treatment with MPP^+ (1 mM) or rotenone (1 μM) for 24 h significantly decreased cell viability as well. (F) 6-OHDA (120 μM), MPP^+ (1 mM) or rotenone (1 μM) increased cleavages of caspase-3 and PARP in PC12 cells and primary neurons. Results are presented as mean ± SEM, n = 5. $^{**}P < 0.01$, difference with control group.