THE ROLE OF ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE IN NEURODEGENERATION IN PARKINSON'S DISEASE

ULOGA PROTEIN KINAZE AKTIVIRANE ADENOZIN MONOFOSFATOM U NEURODEGENERACIJI U PARKINSONOVOJ BOLESTI

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Abstract

Parkinson's disease (PD) is defined as a chronic neurodegenerative disorder with a gradual demise of dopaminergic (DA) neurons in *substantia nigra pars compacta*. Although molecular mechanisms of DA neurons' degeneration are still insufficiently understood, different studies based on animal, toxic and genetic models, as well as post-mortem brain tissue analyses revealed several common features of neuronal death in SN: mitochondrial dysfunction, increased ROS production and impaired proteostasis. Each of these mechanisms might be, at least in part, related to adenosine monophosphate-activated protein kinase (AMPK). The AMPK is a major intracellular energy sensor. It can be activated by different types of metabolic stress, mediated by at least three different kinases LKB1, CAMKKβ, and TAK1, in conditions of decreased cellular ATP and/or increased Ca^{2+} level. Once activated, AMPK promotes catabolic pathways, inhibits mTORC1, and stimulates autophagy, mitochondrial biogenesis, and turnover. Different studies revealed a growing body of evidence suggesting an important role of AMPK in PD. Many of them have documented the protective effect of AMPK activation in different PD models by facilitating mitochondrial quality control, enhancing autophagic clearance of defective mitochondria and protein aggregates. However, some studies have shown the detrimental effect of AMPK activation in DA neurons in advanced stages of neuronal damage, where prolonged activation could inhibit protein synthesis and impair synaptic integrity and plasticity. In this review, we will try to summarize the literature data regarding the role of AMPK in PD pathogenesis.

Sažetak

Parkinsonova bolest (PB) je neurodegenerativno oboljenje koje karakterišu progresivno izumiranje dopaminergičkih neurona (DA) u *substantia nigra pars compacta* (SN). Iako mehanizam koji dovodi do oštećenja DA u PB nije u potpunosti razjašnjen, različite studije na životinjama, toksični i genski modeli, kao i obdukcione analize tkiva mozga pacijenata obolelih od PB pokazale su neke zajedničke osobine umirućih neurona: disfunkcionalne mitohondrije, povišen oksidativni stres i narušenu proteostazu. Svaki od ovih mehanizama mogao bi, barem u nekoj od faza bolesti, da se dovede u vezu sa protein kinazom aktiviranom adenozin monofosfatom (AMPK). To je najvažniji unutarćelijski energetski senzor. U uslovima metaboličkog stresa, kada je smanjena količina unutarćelijskog ATP i/ili usled porasta nivoa jona kalcijuma (Ca^{2+}), AMPK aktiviraju najmanje tri ushodne kinaze: LKB1, CAMKKβ i TAK1. Na taj način AMPK pokreće kataboličke procese, inhibira mTORC1 i stimuliše autofagiju, biogenezu mitohondrija, kao i proces otklanjanja oštećenih mitohondrija iz mitohondrijalne mreže. Brojne studije su pokazale da AMPK ima značajnu ulogu u PB. Pojedine studije ukazuju da bi AMPK mogla biti uključena u protektivne mehanizme u PB, pre svega jer učestvuje u procesima kontrole kvaliteta mitohondrija i stimuliše autofagiju i otklanjanje oštećenih mitohondrija i proteinskih agregata. Nasuprot ovome, neke studije su pokazale da aktivacija AMPK može da ima štetan efekat u DA neuronima u uznapredovalim fazama bolesti usled inhibicije sinteze proteina, što za posledicu ima smanjenje integriteta i plastičnosti sinapsi. U ovom mini-preglednom članku trudićemo se da rezimiramo dosadašnje podatke iz literature o ulozi AMPK u patogenezi PB.

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder characterized by the chronic demise of dopaminergic neurons in the *substantia nigra* (SN) and the presence of intracellular inclusions called Lewy bodies (1–4). Due to the loss of dopaminergic neurons, this disease is characterized by motor symptoms: bradykinesia, static tremor, muscle rigidity and postural instability (5). These motor features may be accompanied by non-motor symptoms, such as a decrease in gastrointestinal motility, loss of olfactory function, sleep dysfunction and/or neuropsychiatric disorders, which seems to occur years before the appearance of clinical manifestations of the disease (6–9).

During the past 40 years, numerous studies of molecular mechanisms involved in PD pathogenesis improved our understanding of events that could lead to PD. Today we know that certain genes and environmental factors could contribute to disease development. Key molecular mechanisms that appear to be relevant for the development and progression of PD are oxidative stress, mitochondrial dysfunction, and altered proteostasis. Each of these mechanisms is, at least at some point, regulated by the enzyme adenosine-monophosphate activated protein kinase (AMPK).

The AMPK is a principal intracellular energy sensor that is activated by different types of metabolic stress that often include an increase in cellular AMP, ADP or Ca^{2+}. It regulates metabolic homeostasis by stimulating catabolic processes while inhibiting anabolic ones (10). Furthermore, AMPK is a major inhibitor of mTORC1 (mechanistic target of rapamycin complex 1), another serine/threonine kinase and the main inhibitor of autophagy (11).

Compromised cellular energy homeostasis, such as disturbances in AMP/ATP ratio, is seen in patients with PD (12). Taking into account that neurons exhibit extraordinarily high energy demands, they are very vulnerable to impaired cellular energy metabolism, suggesting the importance of AMPK in the maintenance of neuronal functioning. This review will briefly summarize several functions of AMPK and their potential relevance to PD.

Molecular mechanisms involved in PD

Experimental studies with neurotoxin-based cellular and animal models and the discovery of monogenic mutations provided valuable data, which indicated that impaired protein degradation systems and Ca^{2+} homeostasis, oxidative stress and mitochondrial dysfunction, could lead to dopaminergic neurons' cellular dysfunction and cell death (7,13) (**figure 1**).

Phenotypes consistent with sporadic PD can be induced by a number of inhibitors of mitochondrial function, such as rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), pesticide paraquat, the dopamine metabolite aminochrome and others (14). It was observed that occupational exposure to pesticides and heavy metals is a risk factor for developing PD (15).

Genetic research in PD have led to the identification of at least 23 gene mutations in which parkinsonism, as a clinical syndrome, is a consistent/dominant feature (16). These include both autosomal dominant and recessive gene mutations (**table 1**) (17). Many of these gene loci are related to mitochondrial functioning. In addition, Genome-Wide Association Studies (GWAS) pointed out that polymorphisms in these genes could be a risk factor for disease development (18).
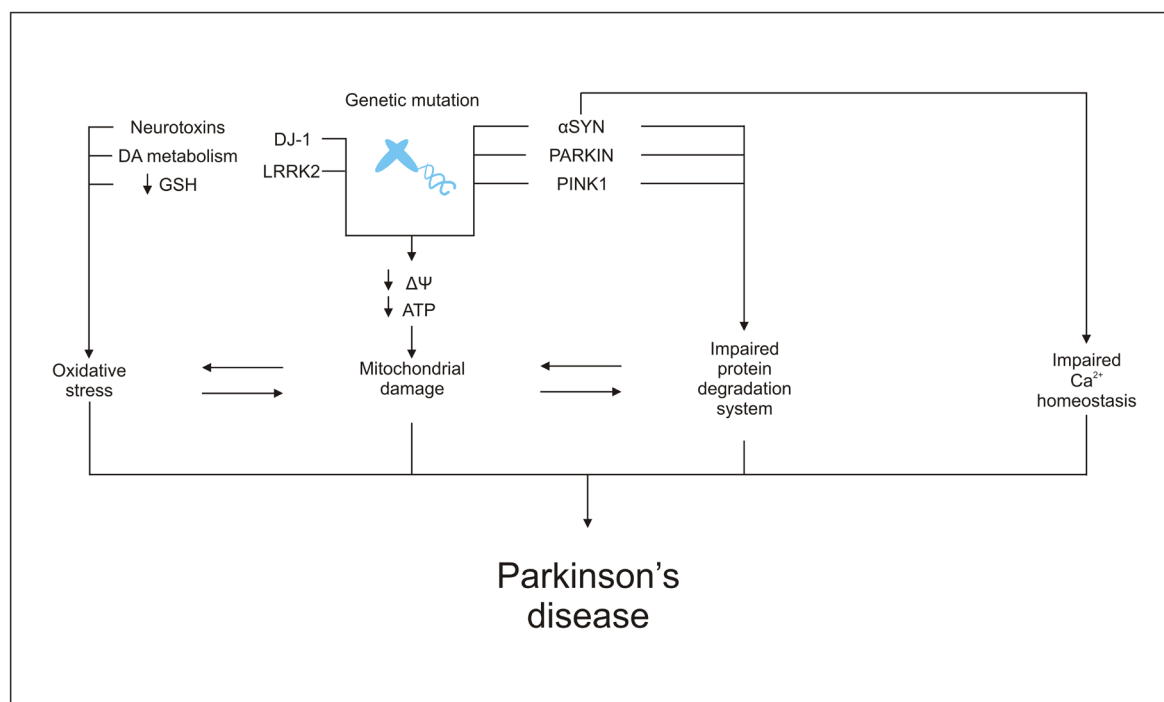
Figure 1. Molecular mechanisms of PD
Impaired protein degradation systems and Ca^{2+} homeostasis, elevated ROS production and mitochondrial dysfunction, are key events in PD neurodegeneration. High-energy demands of DA neurons, DA metabolism, decreased levels of antioxidants in SN made dopaminergic neurons more susceptible to oxidative stress and neurotoxin effects. Genetic studies revealed genes linked to PD–α-synuclein, LRRK2, DJ-1, PINK1, and Parkin. These mutations may directly induce mitochondrial damage and oxidative stress. Also, it was discovered that α-synuclein, a major component of Lewy bodies, could play a role in impaired Ca^{2+} homeostasis.

Table 1. The most common gene mutations in familial forms of PD

Autosomal dominant gene mutations	Autosomal recessive gene mutations
α-synuclein	Parkin (E3 ubiquitin-protein ligase parkin/PARK2)
UCH-L1 (ubiquitin carboxyl-terminal hydrolase isoenzyme L1/PARK5)	PINK1 (PTEN-induced putative kinase 1/PARK6)
LRRK2 (leucine–rich repeat serine/threonine-protein kinase 2/PARK8)	DJ-1 (PARK7)
	ATP13A2 (PARK9)
	HTRA2 (high-temperature requirement protein A2/PARK 13)

Oxidative stress in PD

At the cellular level, both familial and sporadic PD can be related to oxidative stress (19). Considerable evidence suggests reactive oxygen species (ROS) is one of the major contributors of dopaminergic cell loss in PD. They are regarded as cellular metabolism by-products, formed both in physiological and pathological conditions (20). They also have a high affinity to react with macromolecules and lipids in the cell and to induce oxidative stress. Oxidative stress is best described as a disturbance in the ratio of ROS production and cellular antioxidant capacity (21).

Dopamine (DA) is a catecholamine neurotransmitter synthetized from tyrosine by tyrosine hydroxylase (TH) and DOPA decarboxylase, and stored in synaptic vesicles (5). DA is metabolized *via* monoamine oxidase (MAO), with H_2O_2 as one of the by-products of this reaction (22). H_2O_2 can then enter Fenton's reaction with Fe^{2+} to form highly reactive and toxic hydroxyl radical (23,24), contributing, together with decreased glutathione concentration and antioxidant

enzymes activity, iron accumulation, etc, to DA neurons' high vulnerability to oxidative stress (19).

Post-mortem brain-tissue analyzes revealed that PD patients had decreased levels of glutathione (GSH) and increased levels of iron in SN, compared to controls (25–27).

Mitochondrial dysfunction in PD

Mitochondria are organelles surrounded by a double-membrane system, with their own DNA (mtDNA). Mitochondria generate energy *via* aerobic oxidative phosphorylation, and actively participate in important cellular functions such as Ca^{2+} homeostasis and signaling, modulation of apoptosis, etc. (28). The inner mitochondrial membrane contains five protein complexes of electron transport chain (ETC), responsible for mitochondrial respiration and ATP production. However, electron transfer in the ETC can result in the formation and release of ROS (29–31). The mtDNA is especially sensitive to ROS damage since it is located in close

proximity to ETC, without histones and with limited DNA repair mechanisms, compared to nuclear DNA (32).

The idea that oxidative stress and mitochondrial dysfunction could contribute to the development of PD emerged from publications showing that accidental application of complex I blocker MPTP mimicked symptoms of PD (33,34). Different toxin-induced models, based on the application of MPTP/MPP+, 6-hydroxydopamine (6-OHDA), rotenone or paraquat provided valuable information about the molecular events involved in DA neurons' demise, as all of these mitochondrial toxins induced mitochondrial dysfunction, oxidative stress, leading to cell death of dopaminergic neurons (36-40). The evidence that mitochondrial complex I activity is decreased in post-mortem SN samples of patients suffering from a sporadic form of PD has further supported these findings (35).

Altered proteostasis in PD

The defining cellular neuropathological hallmark of PD are Lewy bodies (3). Their main component is α-synuclein and its abnormal filamentous assemblies (36). The α-synuclein gene locus multiplications and missense mutations have been associated with the familial PD (37,38), while polymorphisms in α-synuclein gene locus have been associated with susceptibility to sporadic PD (39). Both ubiquitin-proteasome system (UPS) and lysosomal protein degradation pathways macroautophagy (referred to hereafter as autophagy) and chaperone-mediated autophagy (CMA), play an important role in α-synuclein clearance (40). Autophagy is an evolutionary conserved lysosome-dependent process, whose main role is the removal of damaged organelles and protein aggregates into lysosomes and their degradation. Many studies demonstrated that α-synuclein clearance by UPS and CMA is impaired in PD (as α-synuclein oligomers inhibit these processes); however, there is also evidence of macroautophagy impairment in PD (41).

Role of AMPK in PD-related neurodegeneration

Structure and regulation of AMPK

The AMPK is an evolutionary conserved serine/threonine kinase and a primary intracellular energy sensor. It consists of catalytic α-subunit and two regulatory subunits, β and γ (10). In conditions of decreased ATP/AMP ratio, AMP induces conformational change of AMPK by binding to its γ subunit. This conformational change promotes the phosphorylation of catalytic α-subunit's Threonine 172 (Thr172) and increases the AMPK catalytic activity by 10-fold (42). It is activated by at least three upstream kinases: the liver kinase B1 (LKB1), Ca^{2+}/calmodulin-dependent kinase β (CAMKKβ) and TGF-β-activated kinase1 (TAK1) (42,43) (**figure 2**). Once activated, AMPK triggers catabolic pathways and represses anabolic pathways in order to maintain energetic homeostasis.

Oxidative stress and AMPK

The energy imbalance that can arise from pro-oxidant conditions in the cell is probably the main mechanism by which increased ROS generation induces the activation of AMPK *in vivo*. However, moderate hypoxic conditions can also initiate ROS-dependent AMPK activation, even without pronounced changes in the adenylate pool (44).

It has been shown that AMPK activation can decrease intracellular ROS level by increasing mitochondrial quality control, and by activating PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) and FOXO3 (Forkhead box transcriptional factor 3) transcription factors increasing transcription of antioxidant enzymes and uncoupling protein 2 (UCP2) (45,46). It seems that activation of AMPK may be the plausible strategy to reduce oxidative stress in DA neurons (47).

Role of AMPK in maintaining cellular energy homeostasis in DA neurons

Cellular energy metabolism impairment has been observed in brain samples from PD patients with both sporadic and genetic forms of the disease (12). Neurons, in general, have high energy demands, mostly required to maintain the ion gradient across their plasma membranes, and, as they lack the capacity to store glycogen, they rely on neighboring astrocytes to provide additional glucose from glycogen breakdown. The DA neurons in SN are more vulnerable to energy stress than neurons in other regions, due to their high energy demands and the fact they are surrounded with only few astrocytes, which are incapable to provide sufficient amount of supplementary glucose. Also, DA neurons have long unmyelinated axons with Ca^{2+} channels, which further contributes to a higher risk for oxidative stress (42). During energy stress, AMPK promotes ATP synthesis *via* mitochondrial biogenesis and inhibits anabolic processes *via* mTORC1 inhibition through activation of hamartine (TSC1 – tuberous sclerosis 1)/tuberin (TSC2 – tuberous sclerosis 2) complex (**figure 2**) (48–50).

Regulation of autophagy by AMPK in neurons

In neurons, autophagy plays a very important role. Damaged mitochondria and protein aggregates are being removed *via* mitophagy (autophagic mitochondria degradation) and CMA.

Autophagy is a coordinated process controlled by several protein complexes (**figure 2**). In conditions of cellular energy stress, AMPK promotes autophagy *via*:

1. Direct phosphorylation and reduction of mTORC1 activity, major autophagy inhibitor (51–53);

2. Initiation of autophagosome formation through phosphorylation of Unc-51 like autophagy activating kinase 1 (ULK1) (54–57); ATG9 (58,59) and Beclin 1-VPS34-VPS15-ATG14L-Atg101 complex (60,61); and

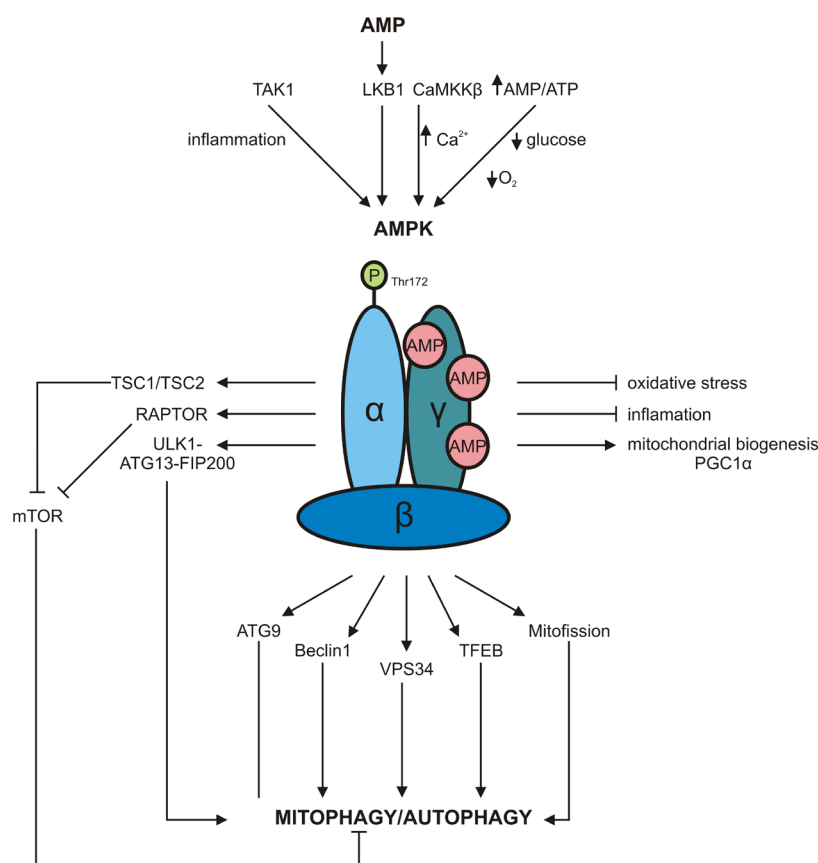3. Increased activity of TFEB (Transcription factor EB) which positively regulates lysosomal biogenesis (62)

Figure 2. Role of AMPK in pathogenesis of PD
AMPK can be activated in, at least, three different ways: by metabolic stress *via* LKB1, by excitotoxic and oxidative stresses through TAK1 and/or CaMKKβ-dependent activation. During energy stress, AMPK phosphorylates the mTOR upstream regulator TSC2 and the mTORC1 subunit RAPTOR. These phosphorylations reduce mTOR activity and thus promote autophagy. AMPK promotes autophagosome formation by direct phosphorylation of ULK1, ATG9, Beclin1, VPS34 and increases TFEB activity.

and promotes autophagy-lysosome pathway-related genes (62–64).

It may be concluded that the role of AMPK in autophagy regulation makes it a plausible molecular target to stimulate autophagy.

The role of AMPK in mitochondrial turnover and biogenesis

Mitochondria are organized in a dynamic tubular network in most eukaryotic cells (65).

Damaged and/or defective mitochondria are excluded from the mitochondrial network by a process called mitochondrial fission and selectively degraded *via* lysosome-mediated autophagic process known as mitophagy (28). On the other hand, partially damaged mitochondria are fused together as a form of complementation in order to reduce energy stress (66). Both processes are complex and controlled by GTPases:

1. Mitofusin-1 and -2 (Mfn-1/2) and optic atrophy 1 (OPA1) for mitochondrial fusion (67);

2. Dynamin-1-related protein (Drp1), mitochondrial fission factor (Mff) and mitochondrial fission protein 1 (Fis) for fission.

As mentioned above, mitochondrial stress rapidly activates AMPK. Once activated, AMPK induces mitochondrial fission *via* phosphorylation of Mff, initiates mitophagy *via* phosphorylation of ULK1 and

promotes mitochondrial biogenesis (10,68,69). The AMPK-dependent phosphorylation of PGC1α induces transcription and replication of mtDNA *via* activation of TFAM (mitochondrial transcription factor A) and promotes transcription of mtDNA (70). Also, phosphorylated PGC-1α promotes fusion/fission, mitophagy, and lysosomal biogenesis by activating transcription of Drp1, Mfn1/2, and TFEB (42).

The role of AMPK in neurodegeneration in PD

Since the first neurotoxin studies and discovery of PD related genes, it became obvious that mitochondrial dysfunction is a very important hallmark of PD. However, numerous studies on the role of AMPK in damage and demise of DA neurons in PD have not reported consistent results.

It has been shown that aging, the biggest risk factor for developing idiopathic PD induces a reduction in AMPK-stimulated activity (71). However, in the 6-OHDA model, AMPK activation was reported, and that further metformin-induced AMPK activation contributed to this toxin's detrimental effect (72). The *in vivo* mouse-model based study showed that metformin exaggerated MPTP-induced dopaminergic damage (73). Some α-synuclein overexpression studies showed that AMPK overactivation enhanced α-synuclein oligomers' accumulation and inhibited neurite outgrowth (74). Kand et al. showed that

AMPK hyperactivation in synucleinopathies could trigger dopaminergic neuronal death (75).

However, several recent publications have reported that in neurotoxin-induced models (MPP⁺/MPTP, rotenone) neuronal AMPK activation had a protective effect (76–79). Furthermore, Dulovic et al. showed that AMPK activators, metformin, and AICAR, had a beneficial effect on cell viability against α-synuclein toxicity (80). In addition, autophagy induced by resveratrol-mediated AMPK activation promoted α-synuclein intracellular degradation (81). A recent publication has demonstrated that AMPKα overexpression *in vivo* protects neurons during the early stages of the α-synuclein pathology, by limiting α-synuclein deposition and causing an increase in total mitochondrial mass (82). Furthermore, AMPK activation exerted beneficial effects on primary human neurons treated with α-synuclein fibrils (83).

Evidence of neuroprotective effects of AMPK were provided in other PD-related models as well. *In vivo* study on *D. melanogaster* revealed that AMPK could have a protective role in familial forms of PD with mutations in Parkin or LRRK2 (84). Similar results with resveratrol-induced AMPK activation were obtained in a study with patients' fibroblast with Parkin mutation, showing autophagy induction and that beneficial effect on mitochondrial function (85).

Conclusion

For the past several decades, the discovery of neurotoxins and PD-linked genes have provided valuable information in understanding molecular mechanisms in DA neurons' degeneration in PD. Oxidative stress and protein aggregates surfaced as major common conditions leading to mitochondrial dysfunction in DA neurons in PD. A considerable body of evidence implicated the important role of AMPK in neuromodulation during the process of neuronal degeneration in SN. Altogether findings from different studies are implying that AMPK activation in PD can be either protective or detrimental to neurons. This discrepancy in literature findings could be the result of different models, type of stimulus, the intensity/length of AMPK activation, etc. It appears that the role of AMPK largely depends on the stage of the neuronal damage. In early stages of DA neurons' dysfunction, AMPK activation appears to have beneficial effects, since it could facilitate mitochondrial quality control, enhance autophagy clearance of protein aggregates and damaged mitochondria. However, in the advanced stages of neuronal demise, chronic AMPK activation represses protein synthesis, which could affect synaptic function and eventually lead to neuronal death (47).

Taking into account the important roles of AMPK in neurons, as well as the observed beneficial effects in earlier stages of neuronal demise, modulation of AMPK activity and its downstream targets seems worthy of further investigation as a promising neuromodulation strategy in PD.

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