



Review article

Teaching the basics of repurposing mitochondria-targeted drugs: From Parkinson's disease to cancer and back to Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) and cancer share common mutations in mitochondrial proteins: Parkin and PINK1. The overlapping of genes involved in PD and cancer implies that the two diseases might share a common pathogenic mechanism. There are other compelling rationales for a mechanistic link between these diseases. Mitochondria and autophagy/mitophagy are emerging as therapeutic targets in PD and cancer: Ongoing research in our laboratories has shown that, when administered early, mitochondria-targeted agents afford neuroprotection in preclinical mice models of PD. Also, we discovered that mitochondria-targeted drugs inhibit tumor cell proliferation. We propose that mitochondrial targeting stimulates conservation of cellular energy critical for neuronal cell survival, whereas the energy conservation mechanism inhibits proliferation of cancer cells by depriving the energy necessary for cancer cell growth. We propose a promising drug repurposing strategy involving mitochondria-targeted drugs synthesized from naturally occurring molecules and FDA-approved drugs that are relatively nontoxic in both PD and cancer. These compounds have been shown to induce various cellular signaling pathways for autophagy/mitophagy, anti-inflammatory, and immunomodulatory effects that are implicated as therapeutic mechanisms in PD and cancer.

"All great achievements of science must start from intuitive knowledge—At times I feel certain I am right while not knowing the reason." —Albert Einstein

1. Introduction: Parkinson's disease and cancer—an odd couple

This review addresses the possibility of repurposing mitochondria-targeted drugs that are modifications of natural compounds and FDA-approved drugs for treating Parkinson's disease (PD) and cancer. At first glance, the connection between PD and cancer may seem far-fetched; however, there are compelling rationales that the two diseases are linked from a mechanistic standpoint. PD and cancer share common mutations in mitochondrial proteins: Parkin and PINK1 [1,2]. The overlapping of genes involved in PD and cancer implies that the two diseases might share a common pathogenic mechanism [1,2]. Metformin, one of the most widely used drugs for the treatment of diabetes, is undergoing clinical trials as a therapy in age-related diseases, including neurodegeneration and cancer [3]. Mitigation of inflammatory processes exerts neuroprotective and antitumor effects [4]. Polyphenolic

compounds have been reported to provide neuroprotection and anti-tumor effects in preclinical neurodegenerative and cancer models [5,6]. Numerous studies have previously reported the neuroprotective effects of small molecular weight antioxidants [7,8]. Mitochondria are emerging as a therapeutic target in PD and cancer [9–12]. Genetic studies implicate mutations in genes (e.g., Park2/Parkin, Park6/PINK1) that mediate mitophagy (autophagic degradation of damaged mitochondrial proteins) are observed in recessive juvenile PD [13]. Emerging literature suggests that defects in the mitophagy machinery caused by alterations in Parkin (also Park2) and PINK1 play a role in tumor progression [2]. Dysfunctional or disrupted autophagic machinery has been linked to PD, cancer, and other diseases [14]. Development of drugs targeting autophagy/mitophagy is an intense area of research [15].

Other reasons for targeting mitochondria include the following: The conservation of cellular energy is vital for the survival of neuronal cells. In contrast, cancer cells divide rapidly. A sustained and increased supply of energy to cancer cells is necessary for cancer cell survival. Thus, mitochondria-targeted drugs prolong neuronal survival by conserving mitochondrial energy utilization and inhibit proliferation of cancer cells by depriving them of energy. Development of mitochondria-targeted

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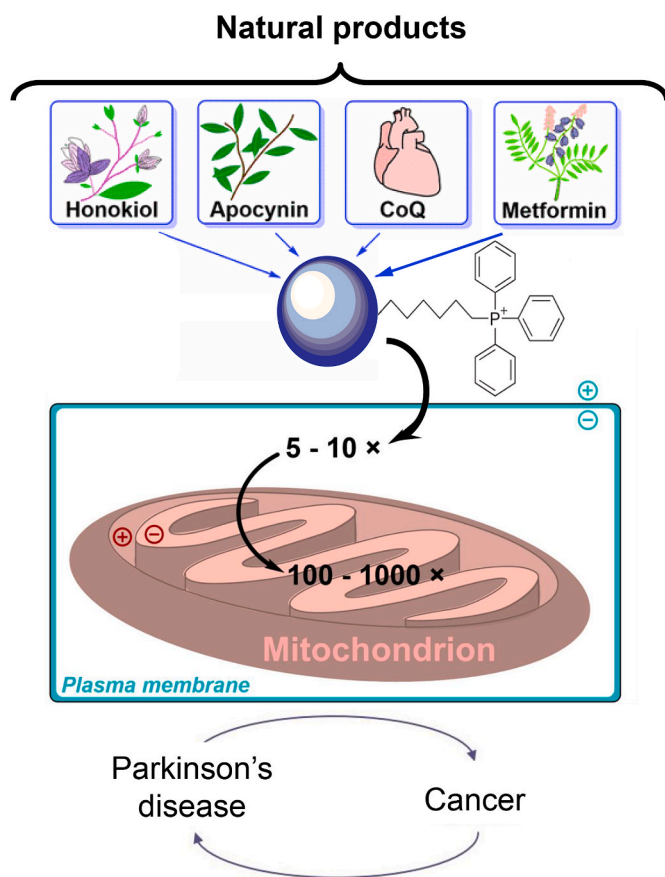


Fig. 1. Increased mitochondrial uptake of alkyl TPP⁺ conjugated to a pharmacophore of selected natural products. Repurposing of these drugs from PD to cancer and back to PD is shown. Adapted with permission from Zielonka J, Joseph J, Sikora A, Hardy M, Ouari O, Vasquez-Vivar J, Cheng G, Lopez M, Kalyanaraman B. Mitochondria-Targeted Triphenylphosphonium-Based Compounds: Syntheses, Mechanisms of Action, and Therapeutic and Diagnostic Applications. *Chem Rev.* 2017; 117 (15):10,043–10120. Copyright 2017 American Chemical Society.

drugs synthesized from naturally occurring compounds and FDA-approved drugs is emerging as a potentially promising approach to investigate the role of mitochondria in diseases [10,16–20]. Fig. 1 shows a list of some of the mitochondria-targeted drugs synthesized from natural products (e.g., Mito-Q, Mito-honokiol, Mito-metformin [Mito-Met], Mito-apocynin [Mito-Apo]). Mitochondria-targeted drugs consist of a triphenylphosphonium (TPP⁺) group attached via varying alkyl chain lengths to a naturally occurring molecule or an FDA-approved drug [17,18,21,22]. As suggested by Murphy and colleagues, a major driving force in targeting these drugs into mitochondria is the delocalized positive charge, its lipophilicity, and the negative plasma membrane and mitochondrial membrane potential (Fig. 1) [10,21–23]. Fine-tuning of TPP⁺-conjugated molecules via an alkyl group of varying chain lengths effectively targets them to mitochondria, thereby minimizing potential off-target effects [18,22]. These molecules are more potent and selective inhibitors of oxidative phosphorylation (OXPHOS), and they lack the toxicity associated with traditional mitochondrial OXPHOS inhibitors (e.g., rotenone, oligomycin) [16,18]. Recently, a *bonafide* OXPHOS inhibitor was approved by the FDA for treatment of multiple myeloma and other hematologic malignancies [24].

2. Targeting mitochondria and mitophagy in PD and cancer

Mitochondrial defects and complex I deficiency are characteristic pathological features of several neurodegenerative diseases including

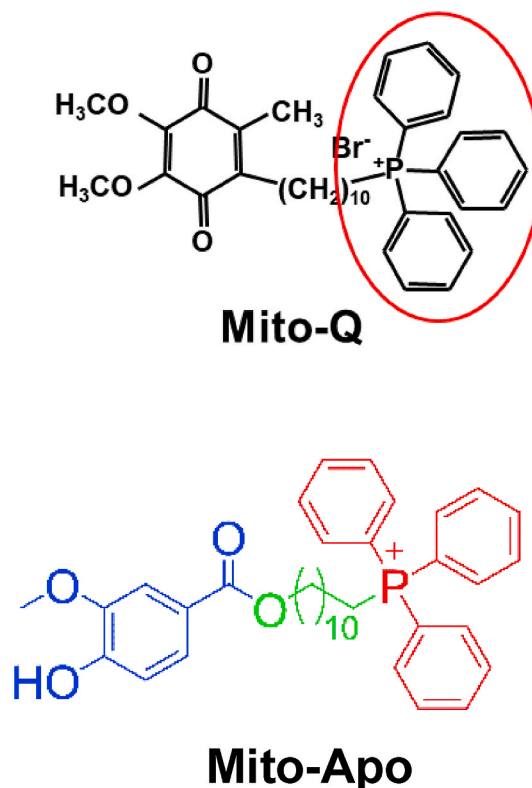


Fig. 2. Structures of Mito-Q and Mito-Apo. Mito-Q is an alkyl TPP⁺ conjugated to Co-Q, and Mito-Apo is an alkyl TPP⁺ conjugated to apocynin.

PD and Alzheimer's disease [25–27]. The activity of complex I associated with the mitochondrial respiratory chain is decreased in PD. This occurs due to sporadic mutations of complex I and/or enhanced oxidative stress in the substantia nigra of the brain. The impaired mitochondria inhibit adenosine triphosphate (ATP) production and decrease bioenergetics. Environmental toxins inhibiting mitochondrial respiration in dopaminergic neurons have been reported to cause a PD-like syndrome [27,28].

Mitophagy is a form of selective autophagy that removes damaged mitochondria through lysosomal degradation and recycling, resulting in healthy mitochondria [29]. Maintenance of healthy mitochondria is achieved through a robust quality control process regulated by mitochondrial biogenesis (i.e., the formation of new mitochondria) and mitophagy, which helps facilitate mitochondrial turnover. Mitophagy therefore plays a critical role in maintaining neuronal function, regulating neuronal survival, and reversing neurodegeneration [30]. Another hallmark of PD was suggested to be defective mitophagy leading to mitochondrial dysfunction [31]. The role of autophagy or mitophagy in cancer is not clear-cut, and inhibitors and stimulators of autophagy exert antitumor effects in a context-dependent manner [32].

Parkin initiates mitophagy and lysosomal degradation of damaged mitochondrial proteins. Parkin encodes a multifunctional E3 ubiquitin ligase responsible for the ubiquitylation of cytosolic proteins other than itself. PINK1 encodes a serine-threonine kinase with a mitochondria-targeting sequence and acts as a molecular sensor of mitochondrial health [29]. PINK1 recruits and activates Parkin upon detection of damaged mitochondria following mitochondrial depolarization [33]. Parkin/PINK1-mediated mitophagy is also induced by mitochondria-targeted agents [34,35]. Parkin activity and enhanced mitophagy have been recognized as tumor suppressor mechanisms. There is connection between energy sensing, adenosine monophosphate (AMP)-activated protein kinase [AMPK] signaling, mammalian target of rapamycin [mTOR] signaling, and the activation of Unc-51-like autophagy-activating kinase 1 (ULK1).

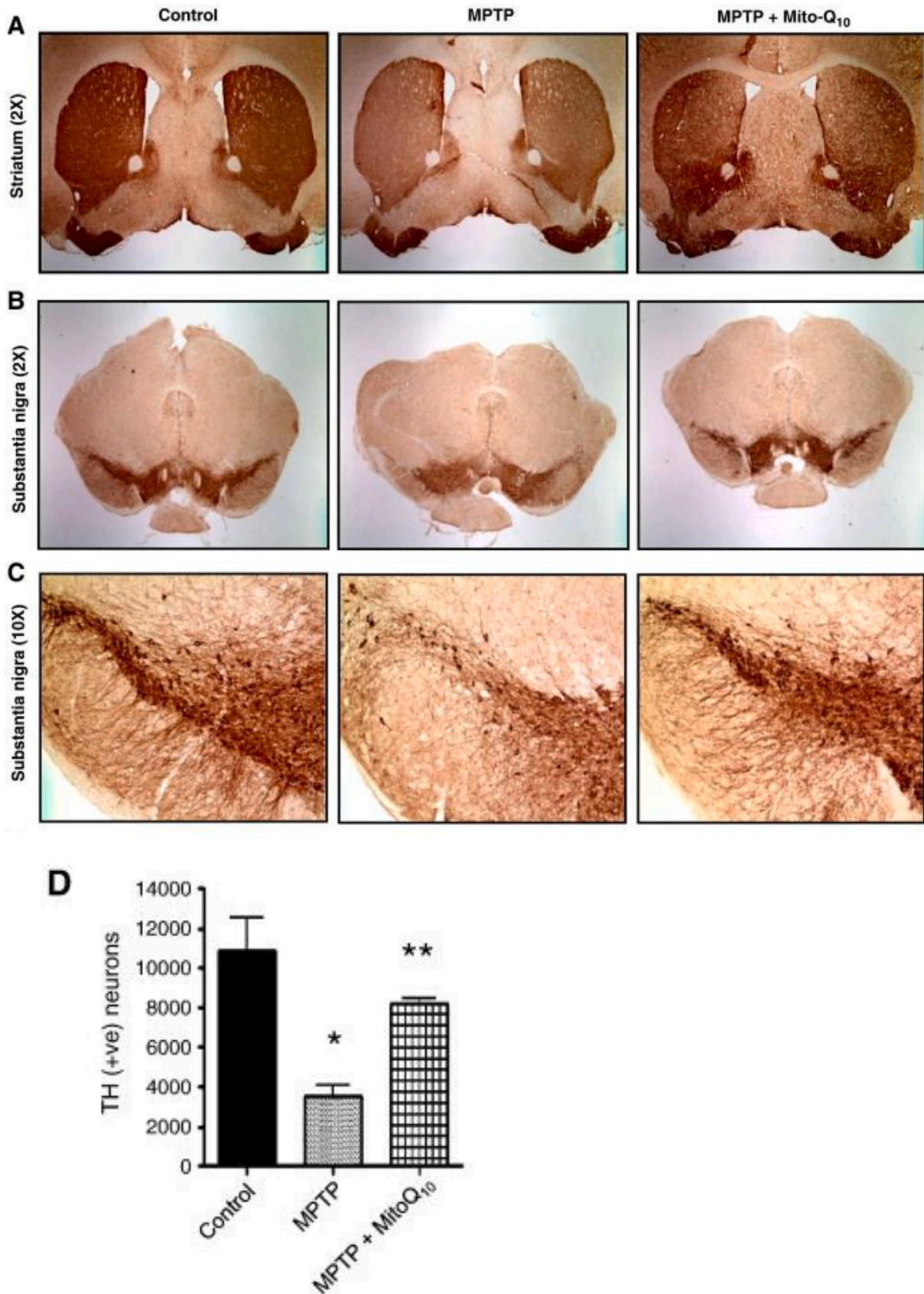


Fig. 3. Mito-Q restores tyrosine hydroxylase-positive dopaminergic neurons in MPTP-treated mice. (A–C) Immunostaining of nigrostriatum and substantia nigra and (D) quantitation of tyrosine hydroxylase-positive neurons in substantia nigra of mice under different treatments. Reprinted from *Free Radical Biology and Medicine*, 49 (11), Ghosh A, Chandran K, Kalivendi SV, Joseph J, Antholine WE, Hillard CJ, Kanthasamy A, Kanthasamy A, Kalyanaraman B, Neuroprotection by a mitochondria-targeted drug in a Parkinson’s disease model, 1674–1684, Copyright 2010, with permission from Elsevier.

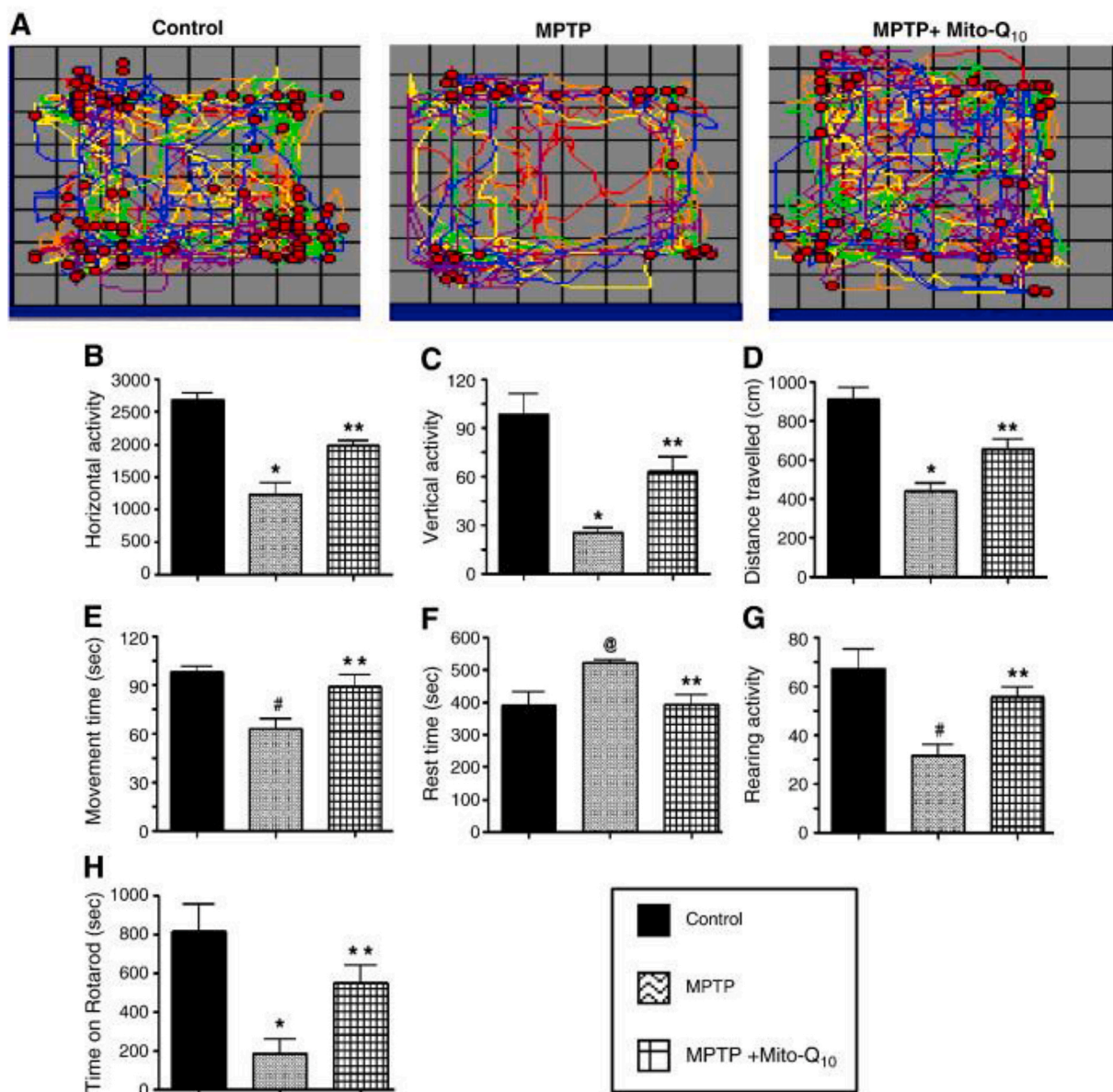


Fig. 4. Mito-Q improves locomotor activity in MPTP-treated mice. (A) Moving track of mice recorded using a VeraMax infrared computerized activity monitoring system. (B–H) Quantitation of locomotor activities (e.g., horizontal activity, vertical activity). Reprinted from *Free Radical Biology and Medicine*, 49 (11), Ghosh A, Chandran K, Kalivendi SV, Joseph J, Antholine WE, Hillard CJ, Kanthasamy A, Kanthasamy A, Kalyanaraman B, Neuroprotection by a mitochondria-targeted drug in a Parkinson’s disease model, 1674–1684, Copyright 2010, with permission from Elsevier.

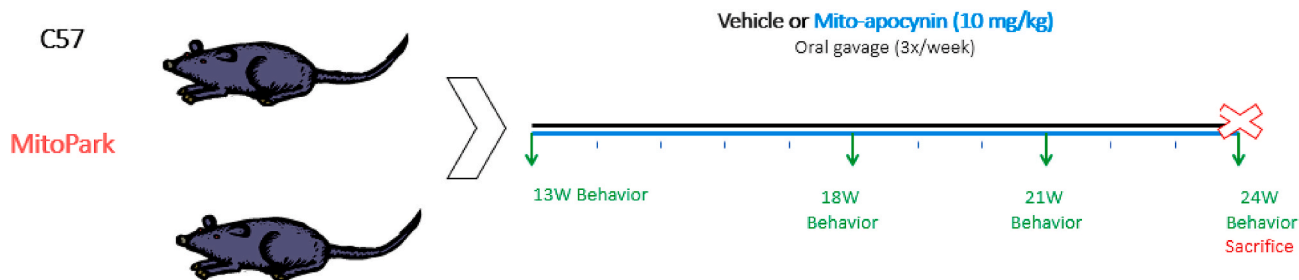


Fig. 5. MitoPark transgenic mice model of PD. (A) Experimental protocol showing administration of vehicle or Mito-Apo in C57 black and MitoPark mice. (B) Time-dependent loss of tyrosine hydroxylase (TH)-positive neurons in MitoPark mice (striatum, substantia nigra). (C) Increased protein occlusions and abnormal mitochondria. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal*. 2017; 27 (14):1048–1066.

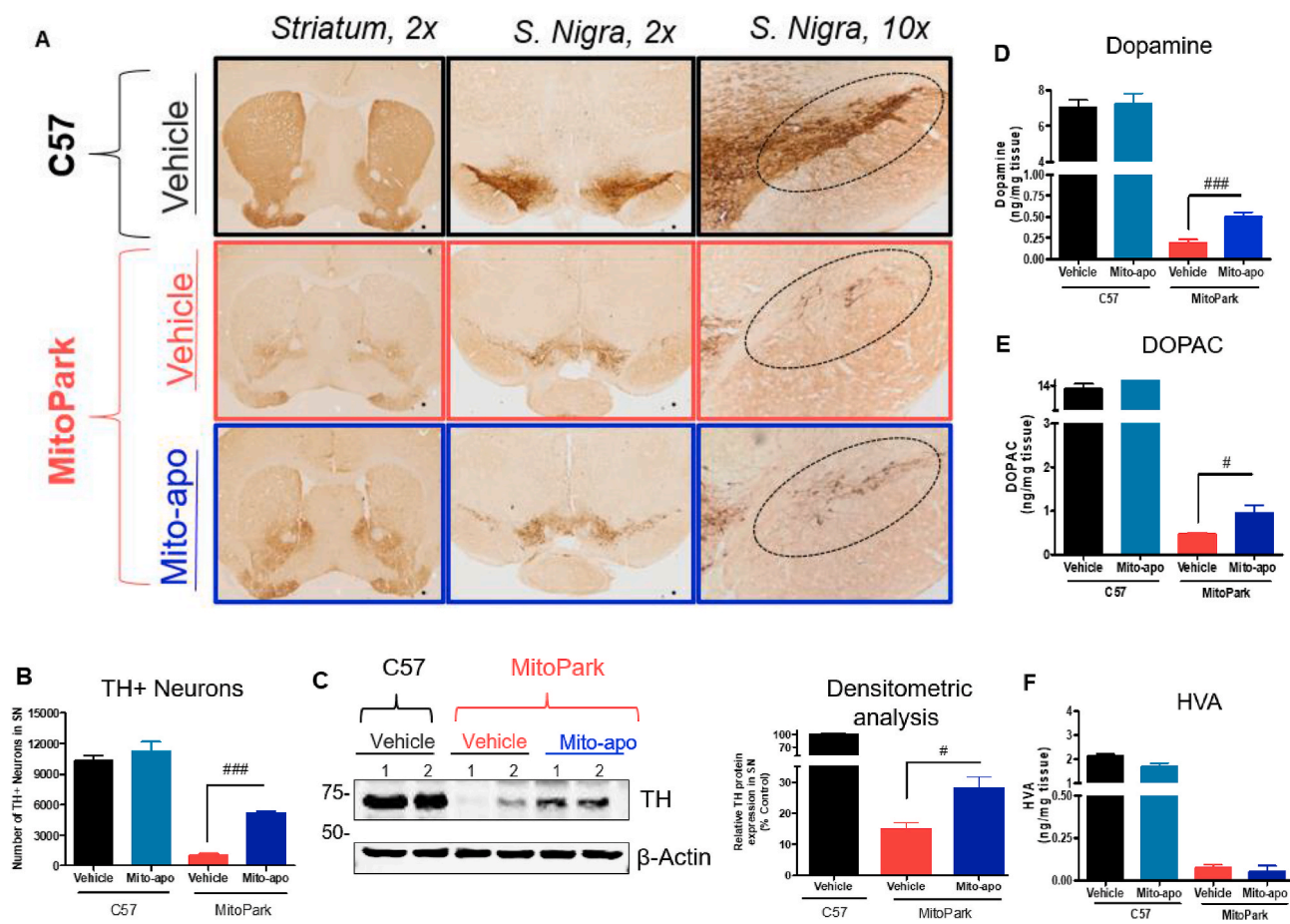


Fig. 6. Neuroprotection by Mito-Apo administration in MitoPark mice. (A) Immunostaining of nigrostriatum of C57 and MitoPark mice administered with vehicle alone and with Mito-Apo. (B) Quantitation of tyrosine hydroxylase-positive (TH⁺) neurons. (C) Western blot showing tyrosine hydroxylase in response to vehicle and Mito-Apo treatment and the densitometric analysis. (D) Quantitation of neuronal dopamine. (E,F) Quantitation of neuronal DOPAC and HVA. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal*. 2017; 27 (14):1048–1066.

The role of mitochondrial function in cancer cells has remained unclear and paradoxical for years. The Warburg effect (*i.e.*, increased glycolysis even under aerobic conditions) was postulated to be the primary—and only—mechanism of ATP and bioenergetics in cancer cells [36,37]. However, recent studies have convincingly demonstrated the functional role of mitochondria in cancer cell proliferation [16–18], and targeting of mitochondria in cancer is emerging as an effective therapeutic strategy [16,18,38]. Parkin plays a role as a tumor suppressor and typically is overexpressed in tumors (glioblastoma and lung cancer). Additional research investigating the link between PD and cancer may provide mechanistic insights into identifying new drugs or repurposing old drugs for treatment of PD and cancer.

3. PD and mitochondria-targeted therapeutics

PD is a progressive and debilitating neurodegenerative disease. Progressive loss of dopaminergic neurons in the substantia nigra of PD patients results in the depletion of dopamine in the striatum. PD is characterized by bradykinesia (slowing physical movement) and rigidity, resting tremors, and postural instability. Loss of smell is one of the earliest symptoms of PD [39]. Another neuropathological hallmark of PD is the aggregation of the alpha-synuclein protein in multiple brain regions [40]. Currently, only the symptoms are treated with dopamine replacement therapy or deep brain stimulation in PD patients. Levodopa (L-dopa) therapy does not reverse or slow down the degeneration of neurons in PD. The exact biological mechanisms of neuronal cell death

in PD remain unknown. At present, no treatment will halt the progression of PD.

Several lines of evidence indicate that free radicals (or reactive oxygen species [ROS]) derived from impaired mitochondrial complex I are involved in PD pathogenesis [41,42]. Enhanced oxidative damage, progressive loss of dopamine, protein nitration, protein inclusions, and Lewy body inclusions consisting of aggregated alpha-synuclein and iron accumulation are characteristic hallmarks of PD pathology [43,44]. Iron chelators and antioxidants had been used with partial success in PD mice models and with little or no success in clinical trials [45,46]. Studies in our laboratory showed that mitochondria-targeted agents (*e.g.*, Mito-CP and Mito-Q) (Fig. 2) mitigate oxidant-induced endothelial cell damage and apoptosis in cell culture systems [47,48]. These mitochondria-targeted agents were not yet used in PD animal models. Therefore, we decided to test the neuroprotective efficacy of Mito-Q10 in a chemically induced mice model of PD [11]. Pioneering research indicated that Mito-Q could potentially act as a mitochondrial ROS detoxifying antioxidant [21,49]. Mito-Q was prepared from modifying the structure of coenzyme-Q (Co-Q) (Fig. 2). We also tested another structurally different mitochondria-targeted agent, Mito-Apo (Fig. 2). Mito-Apo was synthesized from apocynin, a plant-derived molecule that has been used as a nonspecific inhibitor of NADPH oxidase (NOX) enzyme, a major ROS-generating enzyme.

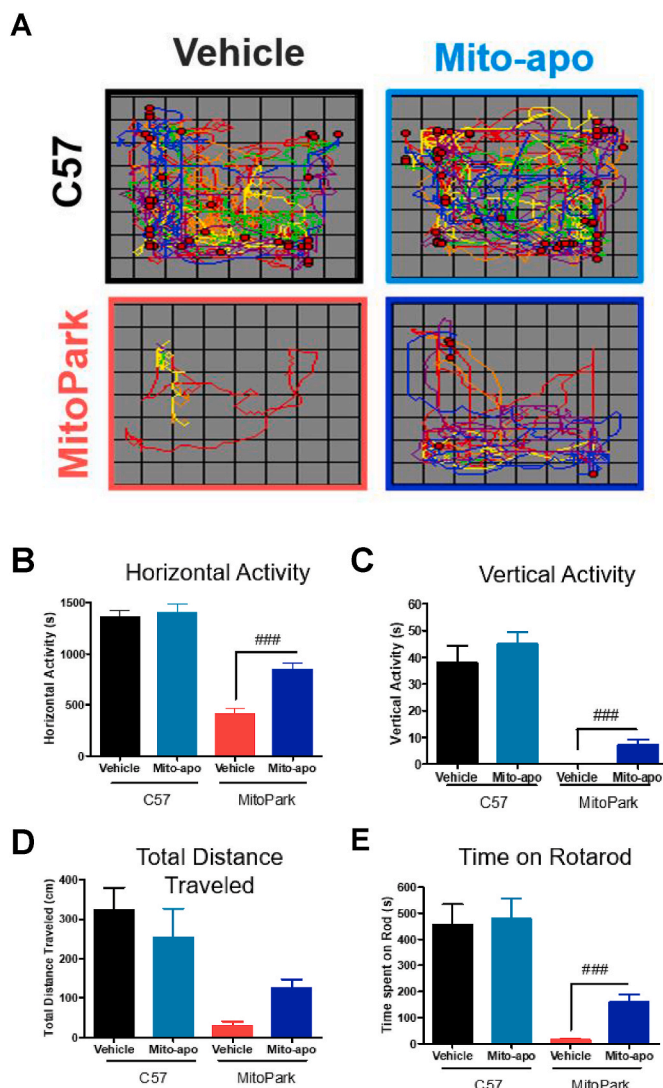


Fig. 7. Mito-Apo improves locomotor activity in MitoPark mice. (A) Moving track of mice in vehicle and Mito-Apo-treated control C57 and MitoPark mice recorded using a VeraMax infrared system. (B–E) Quantitation of locomotor activities. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal*. 2017; 27 (14):1048–1066.

4. Mito-Q and PD

Epidemiological studies suggest a link between mitochondrial toxins (rotenone and pesticides) and the etiology of PD [50]. A commonly used preclinical animal model of PD is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model. MPTP, a contaminant formed during synthesis of an illicit narcotic, induced PD-like symptoms in animals, as noticed in mephedrone (4-methyl ephedrone or M-CAT) drug abusers [51]. Although the chemically induced model of PD differs from idiopathic PD, there are many biochemical and pathological similarities, including the selective destruction of dopaminergic neurons in the substantia nigra. MPTP is highly lipophilic and skin permeable. It is metabolized by monoamine oxidase-catalyzed oxidation to the ultimate toxic metabolite, MPP⁺, that is transported into the dopaminergic neurons and causes neuronal cell death [52].

A substantial decrease in Co-Q, an important cofactor in the mitochondrial electron transport chain, was shown to occur in dopamine neurons of PD patients [53]. Supplementation with high levels of Co-Q

or with a modified and mitochondria-targeted Co-Q (e.g., Mito-Q10) was proposed as a promising neuroprotective strategy [54]. In addition, there was no published study determining the efficacy of mitochondrial-targeted agents in an animal model of PD. Mito-Q10 was suggested to function as an antioxidant through redox cycling between the oxidized (quinone) and reduced (hydroquinone) forms of Mito-Q [55,56]. Our published data show that Mito-Q reversed MPP⁺-induced tyrosine hydroxylase inactivation, decreased dopamine depletion, and decreased caspase-3 activation in dopaminergic cells. Noteworthy findings from these studies are (i) Mito-Q10 treatment protected against MPTP-induced reduction in tyrosine hydroxylase positive neurons (Fig. 3), (ii) Mito-Q10 treatment attenuated dopamine depletion in MPTP-treated mice [11], and (iii) Mito-Q10 protected against MPTP-induced loss of locomotor activities and foot movement (Fig. 4). It is significant to point out that mice were administered Mito-Q before, during, and after treatment with MPTP in these studies. This suggests that prophylactic and continuous administration of Mito-Q10 affords neuroprotection in MPTP-treated mice.

However, a clinical trial administrating Mito-Q10 to PD patients for a year indicated no alterations in disease progression [23]. In humans, Mito-Q10 treatment was started after PD symptoms were observed. As reported, it is likely that nearly 70% or more of dopaminergic neurons were damaged in these patients [57]. The MPTP model, however, does not fully reflect the human PD pathology (e.g., Lewy body inclusion) and does not mimic dopamine loss in the substantia nigra as occurs in age-dependent PD in humans. The timing and dosing of mitochondrial therapeutic intervention are critical for successful treatment of PD. The likelihood of success for therapeutic intervention may be higher in PD patients who are on the verge of developing this dementia. As with treatment of other diseases such as cancer, drug treatment should begin at an early stage of PD. Clearly, discovering early biomarkers of PD that are reliable and specific is urgently needed to enhance therapeutic efficacy.

5. Neuroprotection of Mito-Apo in a preclinical MitoPark mouse model of PD

Apocynin is a naturally occurring plant-derived compound that has shown anti-inflammatory effects in several diseases [58]. Apocynin is also a nonspecific inhibitor of NOX enzymes that generates proinflammatory oxidants involved in inflammation. We modified the structure of apocynin by conjugating it to a TPP⁺ group such that the resulting molecule, Mito-Apo, is targeted to mitochondria (Fig. 2). Mito-Apo exhibits neuroprotective mechanism in a chemically induced Parkinsonian model (MPTP mouse model) [12]. A genetically engineered mouse model, the MitoPark mouse, recapitulates many of the phenotypic features (mitochondrial dysfunction, microglial activation, dopaminergic degeneration and depletion of dopamine, progressive neuronal deficits, and protein occlusion) of human PD [12]. This mouse model has been used to test the hypothesis that mitochondrial dysfunction in dopaminergic neurons is causally responsible for the PD-like phenotype. The MitoPark transgenic mouse is based on the direct genetic impairment of the mitochondrial respiratory chain function in dopamine neurons and was created by selectively knocking out the mitochondrial transcription factor A in dopaminergic neurons [59]. Fig. 5 shows the progressive motor deficits in MitoPark mice. By week 12, the MitoPark mouse began to exhibit behavioral deficits that progressively became worse by week 24. This phenotype also exhibited neurochemical alterations (dopamine depletion) similar to MPTP treated C57 mice. MitoPark mice also exhibited neurochemical alterations similar to those observed in MPTP-treated C57 mice.

MitoPark and age-matched C57 mice were orally administered with vehicle or Mito-Apo beginning at age 13 weeks [12]. Behavioral performances, such as the open-field activity and rotarod activity, were monitored in Mito-Apo and vehicle-treated MitoPark mice. As shown in Fig. 6, Mito-Apo significantly restored the progressive motor deficits

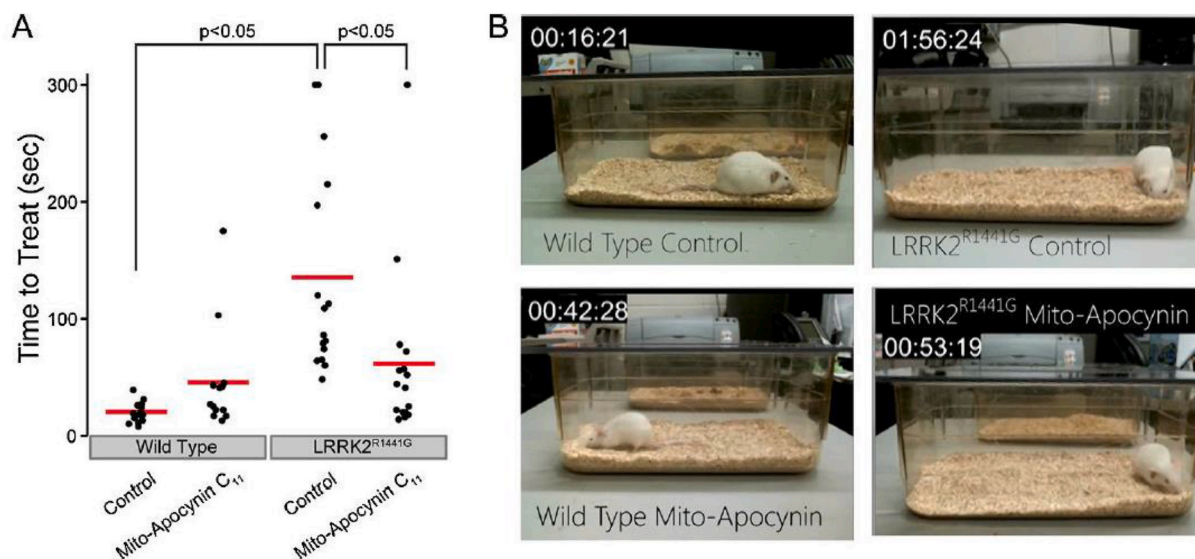


Fig. 8. Mito-Apo prevents loss of smell in LRRK2^{R1441G} transgenic mouse model. (A) The time required to identify either a chow pellet or fruit cereal treat in a novel cage. Individual mice are represented by black dots and the mean is shown as the red bar. (B) Video still images indicate the mouse that had the time-to-treat closest to the group mean. Time stamps indicate the elapsed time required to locate the treat. Reprinted from Neuroscience Letters, 583, Dranka BP, Gifford A, McAllister D, Zielonka J, Joseph J, O'Hara CL, Stucky CL, Kanthasamy AG, Kalyanaraman B, A Novel Mitochondrially-Targeted Apocynin Derivative Prevents Hyposmia and Loss of Motor Function in the Leucine-Rich Repeat Kinase 2 (LRRK2^{R1441G}) Transgenic Mouse Model of Parkinson's Disease, 159–164, Copyright 2014, with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

observed in MitoPark mice (Fig. 7). Mito-Apo treatment inhibited nigrostriatal tyrosine hydroxylase loss, restoring dopamine levels in the striatum of MitoPark mice (Fig. 7). This study reported, for the first time, that an orally active mitochondria-targeted apocynin derivative protected against neurodegeneration in a MitoPark mouse model.

Mito-Apo also mitigated the non-motor symptoms in a PD mouse model. Loss of smell is one of the first signs of PD. Scent testing remains one of the most effective methods for early detection of PD symptoms (hyposmia or diminished loss of smell) in high-risk individuals. We used the leucine-rich repeat kinase 2 (LRRK2) mouse model that is based on elevated LRRK2 kinase activity caused by specific mutations in the LRRK2 gene (mutation of arginine to glycine at position 1441) [60,61]. Long-term treatment with Mito-Apo significantly prevented the loss of smell with performance remaining near the level of the wild-type mice. Prolonged treatment with Mito-Apo improved the delayed time-to-treat in LRRK2^{R1441G} mice (Fig. 8). These studies demonstrate that mitochondria-targeted natural product derivative could be used to prevent early non-motor symptoms.

6. From PD to therapeutic targeting of mitochondria in cancer: our rationale

We decided to test the effects of mitochondria-targeted antioxidants (e.g., Mito-Q, Mito-CP) in cancer cells in order to obtain experimental proof for the functional role of mitochondrial respiration in cancer cells. Warburg hypothesized that cancer cell mitochondria are dysfunctional and, therefore, these cells preferentially use glycolysis for energy even in the presence of oxygen [62–64]. The Warburg discovery (i.e., enhanced uptake of glucose by cancer cells) helped diagnose and detect cancer cells using the PET (positron emission tomography) technique that monitors the selective uptake of radiolabeled FDG (2-fluoro-6-deoxyglucose). However, the proposed lack of functional role of mitochondria in cancer cells by Warburg was criticized and disproved by some and clarified by others [31,36,65]. Using breast cancer cells and non-cancerous control cells, we showed that Mito-Q and Mito-CP, at nontoxic concentrations, selectively inhibit mitochondrial respiration and cell proliferation; this finding clearly proved a functional role for mitochondria in cancer. These studies [17,66] paved the way for

designing new mitochondria-targeted drugs through modification of existing FDA-approved drugs, for example, metformin.

7. Mitochondria-targeted metformin analogs as anti-tumor drugs

Metformin is one of the most widely used and prescribed drugs in the world for treatment of diabetes. Metformin is relatively safe in humans; however, it is poorly bioavailable, and patients with type 2 diabetes mellitus take several grams of metformin daily to decrease blood glucose levels. Metformin is excreted mostly unchanged without undergoing metabolism. However, the efficacy of metformin is attributed to the many metabolic pathways it induces in the body. Metformin is a highly hydrophilic cation at a physiological pH that targets mitochondria, albeit relatively weakly, and inhibits complex I activity in several cancer cells. Metformin was repurposed as an antitumor drug due to epidemiological findings implicating an association between decreased incidence of pancreatic cancer and metformin use in diabetic individuals [67]. Several ongoing clinical trials are testing the antitumor effects of metformin in various cancers including pancreatic cancer. However, at typical antidiabetic doses, metformin is unlikely to exert antitumor effects due to its low bioavailability combined with its low uptake into cancer cells [68]. A more lipophilic and bioavailable analog of metformin, phenformin, was shown to exhibit more potent antitumor effects [69]. Due to enhanced acidosis, phenformin use was discontinued in the United States. Clearly, it is crucial to develop new and improved metformin analogs with increased potency and bioavailability. To this end, mitochondria-targeted metformin analogs were synthesized and developed in our laboratory [18]. Our results showed that Mito-Met analogs are nearly 1000-fold more potent than the untargeted metformin (Fig. 9). Studies with Mito-Met analogs and other mitochondria-targeted compounds provide new insights into the bioenergetic mechanism of action.

8. Mito-Met analogs activate an energy sensing mechanism in cancer cells

Mito-Met10 activated AMPK phosphorylation at micromolar

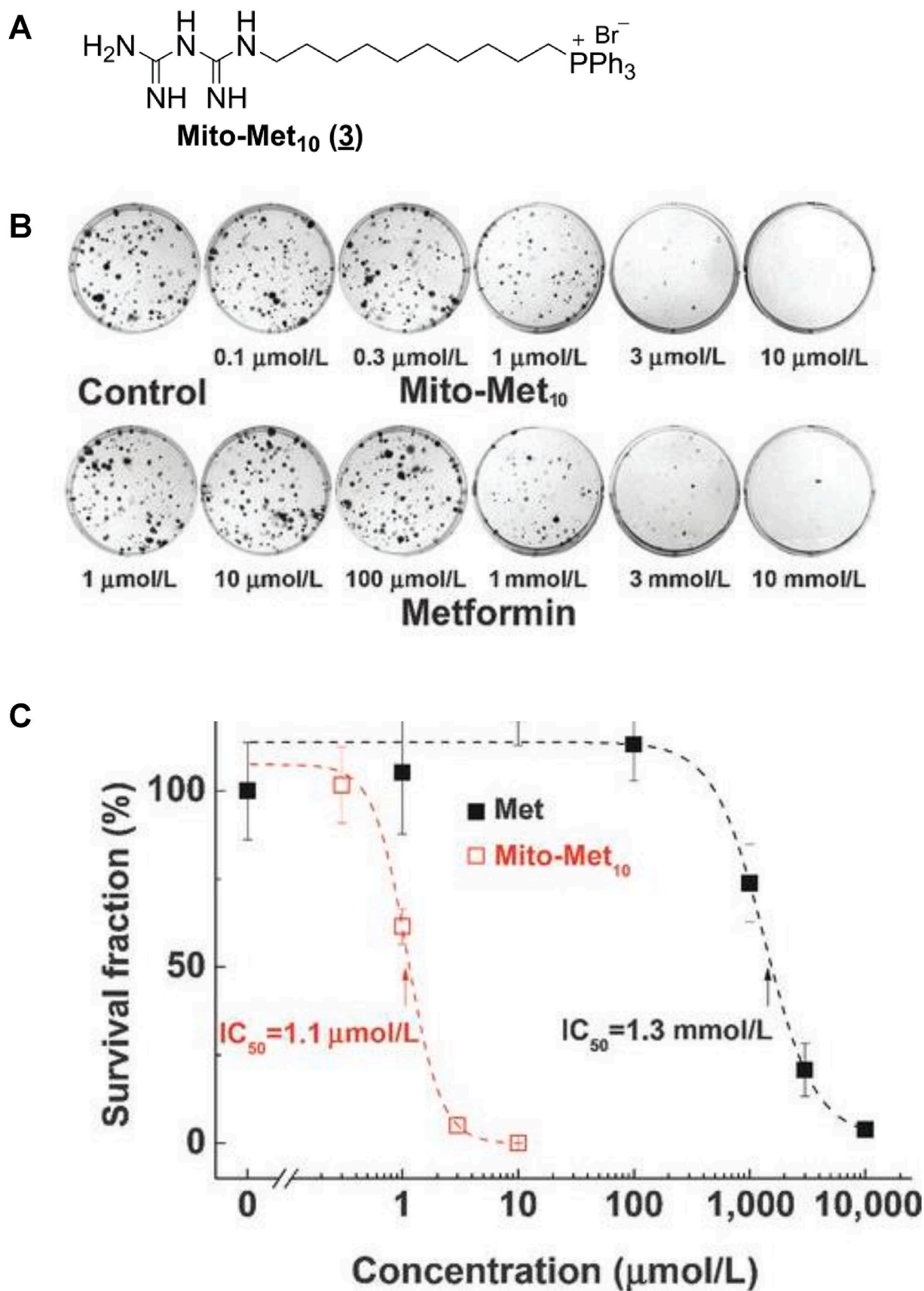


Fig. 9. Effects of metformin and Mito-Met on pancreatic cancer cell proliferation. (A–C) MiaPaCa-2 pancreatic cancer cells were treated with metformin and Mito-Met for 24 h. MiaPaCa-2 cells were treated with metformin and Mito-Met, and colony formation and survival fraction were monitored and calculated for different treatment conditions. Reprinted from Cheng G, Zielonka J, Ouari O et al. Mitochondria-Targeted Analogs of Metformin Exhibit Enhanced Antiproliferative and Radiosensitizing Effects in Pancreatic Cancer Cells. *Cancer Res.* 2016 Jul 1; 76 (13):3904–15.

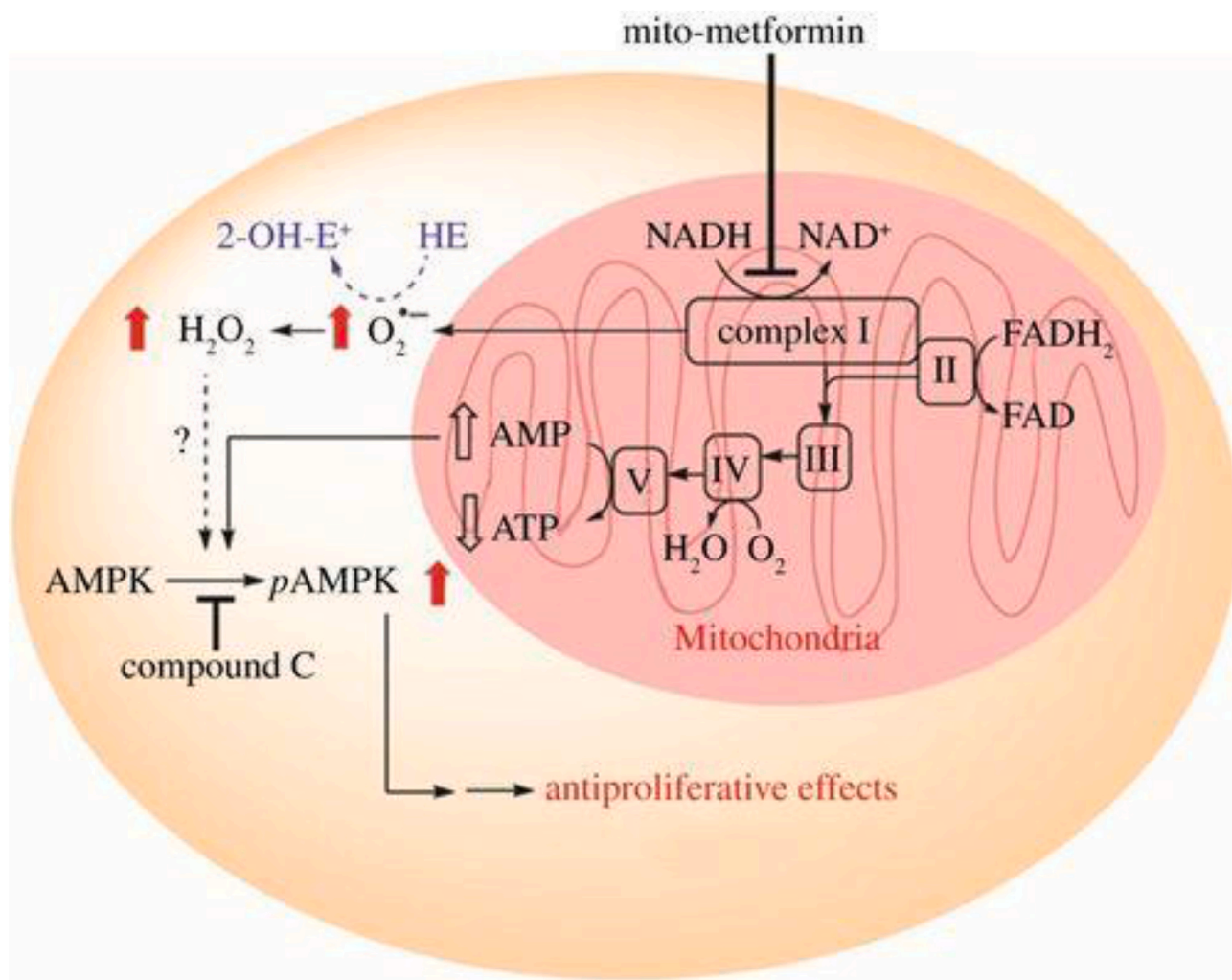


Fig. 10. The proposed signaling pathway activated by Mito-Met and other metformin analogs. As shown, Mito-Met activated AMPK phosphorylation via inhibition of mitochondrial complex I and ROS generation. Reprinted from Cheng G, Zielonka J, Ouari O et al. Mitochondria-Targeted Analogs of Metformin Exhibit Enhanced Antiproliferative and Radiosensitizing Effects in Pancreatic Cancer Cells. *Cancer Res.* 2016 Jul 1; 76 (13):3904–15.

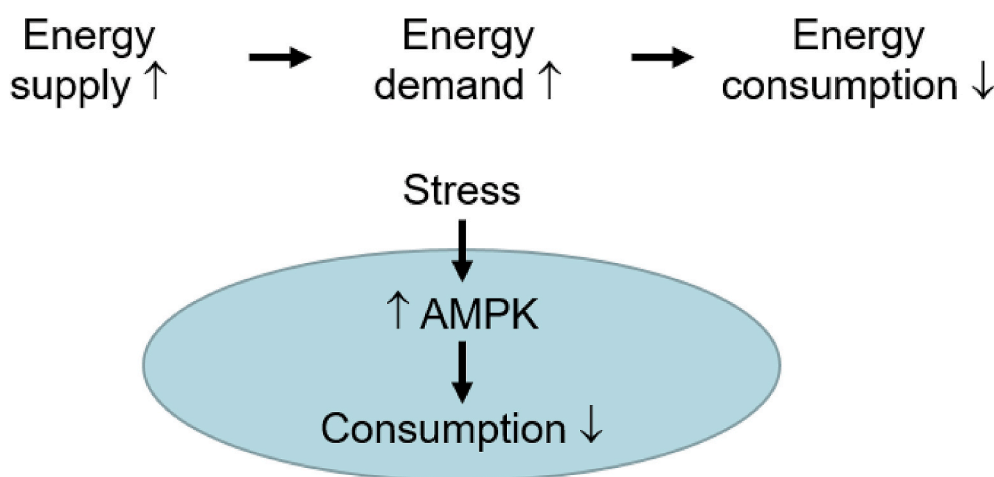
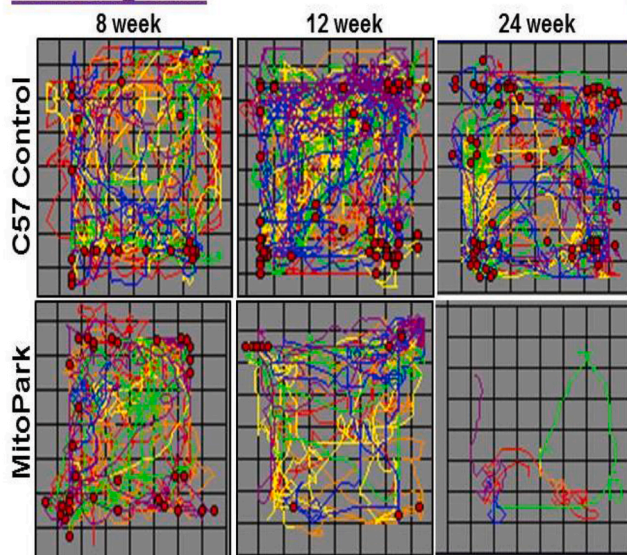


Fig. 11. A proposed pathway illustrating how a mitochondria-targeted drug could affect cell survival based on supply and demand of energy generated in mitochondria. Induction of a mild mitochondrial stress induced by mitochondria-targeting agents inhibits energy consumption through elevation of AMPK phosphorylation and decreased ATP consumption. In neurons, cell survival is enhanced, whereas in cancer cells, proliferation is inhibited. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal.* 2017; 27 (14):1048–1066.

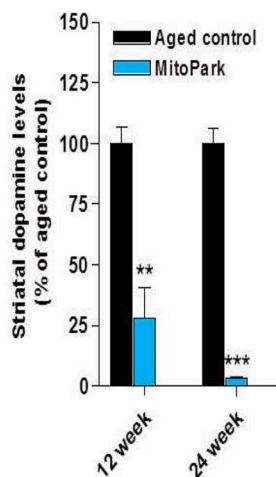
concentrations, whereas metformin activated AMPK at millimolar levels in pancreatic cancer cells [18]. We postulated that Mito-Met10 exerts antiproliferative effects in pancreatic cancer cells by targeting both redox- and energy-sensing bioenergetics pathways (Fig. 10). Factors

responsible for AMPK activation need to be fully ascertained, although mitochondrial ROS may be involved [18]. Mitochondria-targeted compounds could induce a novel redox-signaling mechanism in which hydrogen peroxide may play a role in the antiproliferative effects in

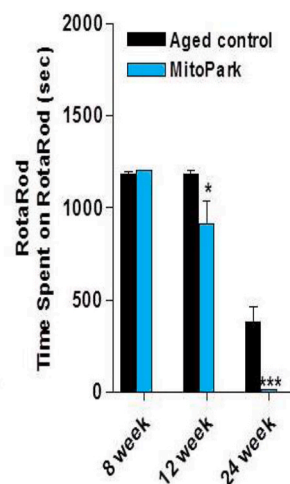
A. Moving track



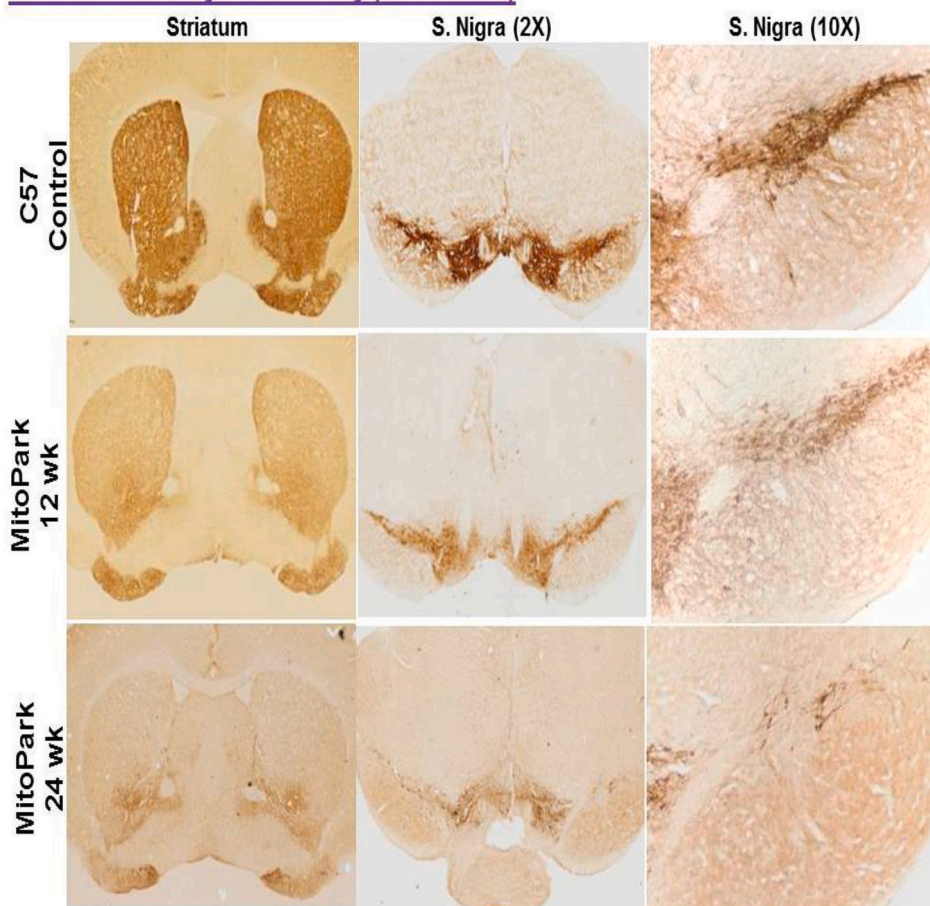
B. Horizontal activity



C. Rotarod performance



D. TH immunocytochemistry (DAB stain)



E. Depletion of striatal dopamine

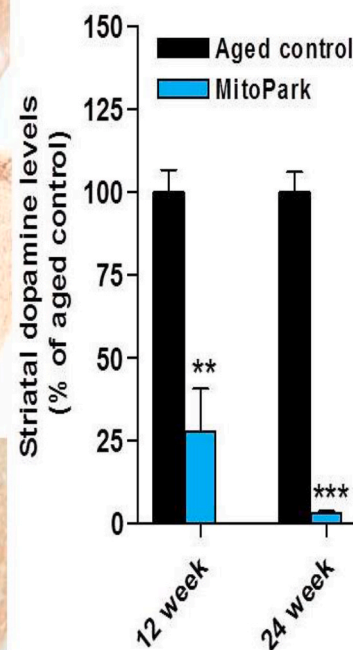


Fig. 12. Neuroprotection by Mito-Met in a MitoPark mice model of PD. (A) Moving track of MitoPark mice recorded using a VeraMax infrared computerized activity monitoring system. (B,C) Quantitation of locomotor activities (e.g., horizontal activity, vertical activity). (D) Immunostaining of nigrostriatum of MitoPark mice administered with vehicle or Mito-Met. (E) Quantitation of neuronal dopamine.

cancer cells [18]. However, mechanistic studies using cancer cells with enriched mitochondrial hydrogen peroxide detoxifying enzymes (e.g., peroxiredoxins) and AMPK signaling pathways are still lacking. Overall, these studies suggest that mitochondria-targeted agents inhibit cancer

cell proliferation by decreasing intracellular energy consumption as cancer cells require increased energetic requirements to sustain enhanced energy demands due to uncontrolled cell proliferation.

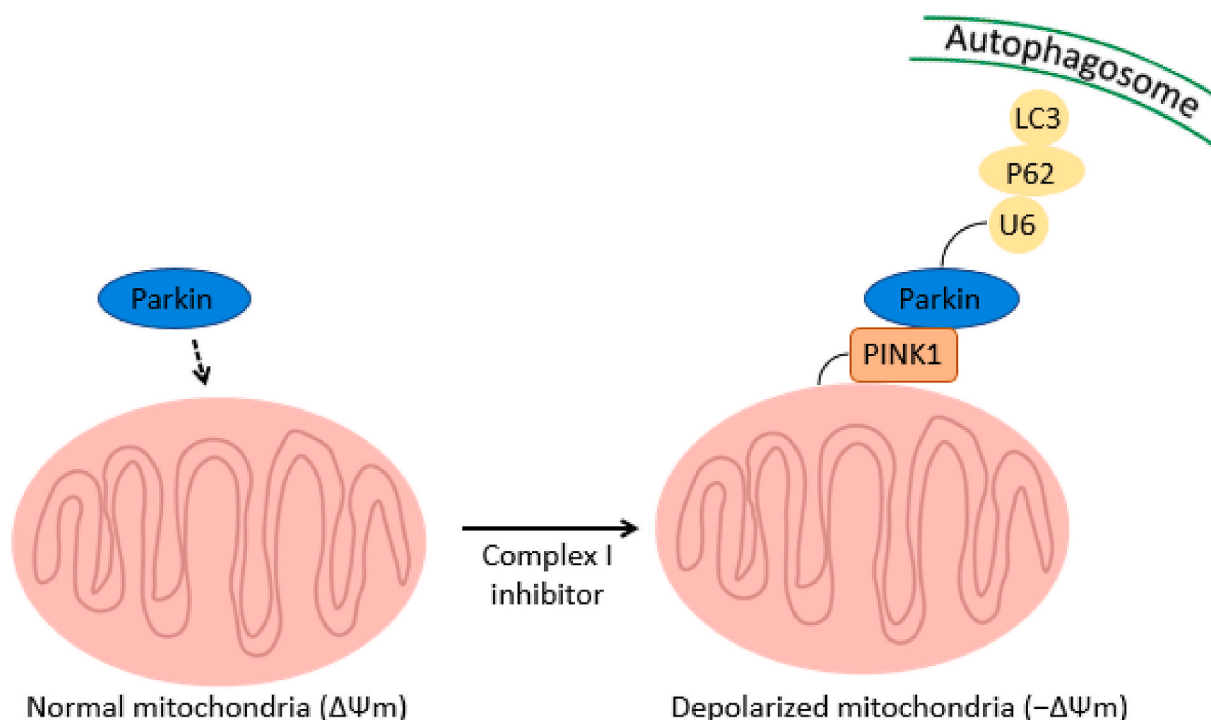


Fig. 13. Parkin/PINK1-mediated pathway in stimulation of autophagy in PD. Mitophagy activated by mitochondria-targeted drugs decreases the mitochondrial membrane potential. PINK1, which normally is processed by mitochondrial peptidase, accumulates at the outer mitochondrial membrane and triggers the recruitment of Parkin, which initiates the autophagic machinery. Adapted from Molecular and Cellular Neuroscience, 55, Vries RLA, Przedborski S. Mitophagy and Parkinson's Disease: Be Eaten to Stay Healthy, 37–43, Copyright 2013, with permission from Elsevier.

9. From cancer back to therapeutic targeting of PD: our rationale

Based on the findings from Mito-Met in cancer cells, we surmised that mitochondria-targeted drugs could enhance neuronal survival because of decreased energy consumption from AMPK activation. Fig. 11 illustrates this idea. AMPK activation through decreased ATP and increased AMP creates low-energy conditions because of decreased energy consumption. It is conceivable that induction of mild mitochondrial stress by Mito-Met could increase neuronal cell survival. Mitochondria-targeted drugs are different from rapamycin analogs that show neuroprotective effects by inhibiting mTOR. Rapamycin and its analogs (also referred to as “Rapalogs”) directly inhibit mTOR, whereas mitochondria-targeted drugs indirectly inhibit the mTOR pathway through inhibition of mitochondrial respiration [3].

10. Neuroprotection by Mito-Met in a MitoPark mouse model

Studies show that metformin reversed TRAPS (TNF receptor-associated periodic syndrome)-mutation-associated alterations in the mitochondrial function in PD mice models [70]. Because metformin also induced AMPK at higher concentrations and induced neuroprotection, we decided to investigate the neuroprotective effect of Mito-Met in a chronic and progressive neurodegenerative MitoPark transgenic mouse model. The motor and non-motor behavioral effects and neurochemical analyses (striatal dopamine) were measured. Mito-Met treatment improved behavioral and neurochemical deficits *in vivo* (Fig. 12). Dopamine levels were partially restored.

More-recent studies show that other mitochondria-targeted compounds (Mito-Q) also stimulated AMPK activation [71], suggesting that the neuroprotective effects of Mito-Q could be attributed to its ability to decrease energy consumption in cells.

These findings show that a new class of mitochondria-targeted drugs that modulates mitochondrial bioenergetics is therapeutically effective

in both PD and cancer. However, the common mechanism of action for the different mitochondria-targeted compounds in PD and cancer is not known. The neuroprotective and antitumor mechanisms of Mito-Q were previously attributed to detoxification of ROS [11,17]. However, Mito-Met inhibits mitochondrial complex I and induces ROS formation in tumor cells [18]. The neuroprotective mechanism of Mito-Apo was attributed to inhibition of the NOX enzyme and inducible nitric oxide synthase (NOS) [12,19].

Recent reports indicate that indicate that Mito-Q activates AMPK signaling via depolarization of mitochondria and induction of autophagy, promoting cell survival [71]. Mito-Q induced autophagy in hepatocellular carcinoma HepG2 cells via altered mitochondrial bioenergetic pathways. Mito-Q enhanced AMPK phosphorylation and inhibited mTOR phosphorylation. Mito-Q induced mitophagy under conditions activating AMPK [72]. Thus, we started focusing on the mechanism of induction of autophagy by mitochondria-targeted compounds as a common mechanism of action in cancer and neuronal cells.

11. Mitochondria-targeted drugs and induction of autophagy

Yoshinori Ohsumi received the Nobel Prize for uncovering the mechanisms for autophagy [73], a fundamental process in cells that can be harnessed to fight cancer and dementia. Autophagy is a “self-eating” process by which damaged intracellular components are degraded and removed by lysosomes. This process is essential to manage stress response (bioenergetic stress, depletion of nutrients, or starvation) and maintenance of tissue homeostasis. Mitophagy is referred to a selective autophagic degradation of damaged or dysfunctional mitochondrial proteins. Although the role of autophagy in PD and cancer remains paradoxical, published reports suggest that autophagic induction could be used as a therapeutic strategy [15]. Recent studies suggest that autophagy is a druggable process [74,75] and that an FDA-approved autophagy-inducing tyrosine kinase inhibitor (nilotinib or Tasigna R) for treating chronic myeloid leukemia can cross the blood-brain barrier

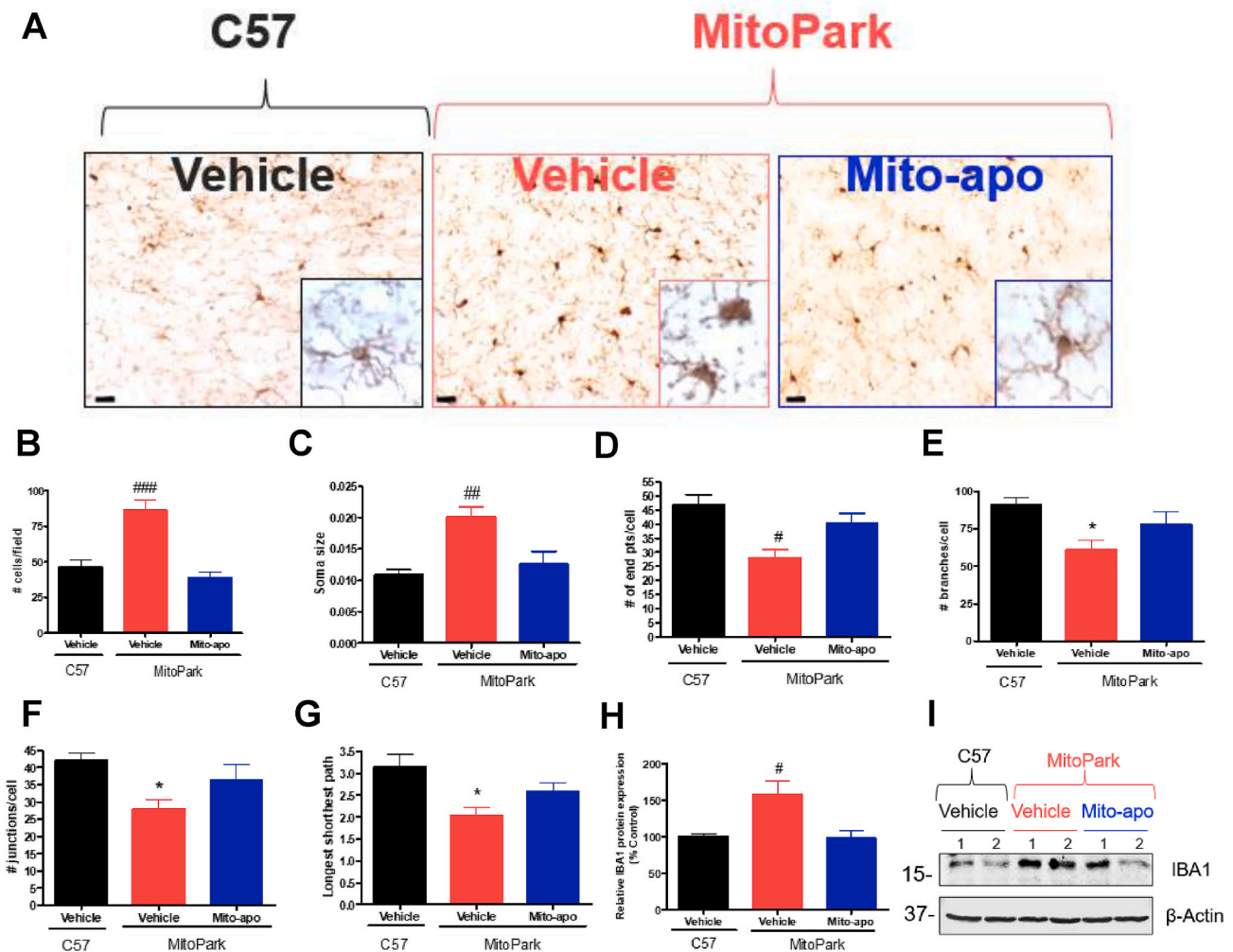


Fig. 14. Mito-Apo inhibits microglial activation in the substantia nigra of MitoPark mice. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal.* 2017; 27 (14):1048–1066.

and remove toxic proteins (beta-amyloid aggregates or Lewy body inclusion) via stimulation of autophagy [76,77]. Nilotinib is currently being tested in PD patients.

Rapamycin is frequently used as a potent inducer of autophagy by directly blocking mTOR (mechanistic target of rapamycin), an upstream inhibitor of the autophagy pathway. mTOR is, however, involved in various biological and developmental processes (translation, ribosome biogenesis). Rapamycin, which is used to treat advanced cancer, has been repurposed as a neuroprotective drug [78,79]. However, the immunosuppressive aspect of rapamycin is linked to higher infection rates. Thus, developing a new class of mitochondria-targeted autophagy-inducing agents that is nontoxic with minimal off-target effects is crucial.

12. Mitophagy and cancer

Recently, we showed that Mito-Met induces mitophagy in cancer cells [16]. Results showed that Mito-Met activates the AMPK signaling cascade triggered by inhibiting mitochondrial complex I. AMPK activation suppressed mTOR. These two signaling pathways stimulate the phosphorylation of ULK1, the central autophagy-inducing kinase [16]. Emerging data show that drugs specifically targeted to mitochondria could stimulate mitophagy in cancer cells [16,72]. The ULK1/AMPK axis is important for regulating the phosphorylation of ULK1 in the

context of glucose or nutrient starvation. Under glucose starvation, the mitophagic signaling mechanism (AMPK/mTOR/ULK1) is activated. Other mitochondria-targeted compounds have recently been found to stimulate mitophagy in cancer cells [72,80].

13. Mitophagy and PD

PINK1, a mitochondrial serine/threonine kinase, is one of the genes linked to PD. In cancer, it acts in concert with Parkin to initiate the mitophagy process. The proposed Parkin/PINK1-mediated mitophagy model is shown in Fig. 13. The Parkin/PINK1 pathway is responsible for mitochondrial quality control [81]. Deficiency in Parkin or PINK1 function results in the accumulation of damaged mitochondrial proteins. In healthy mitochondria, PINK1 does not accumulate in the outer membrane. In damaged or depolarized mitochondria, PINK1 stabilization on the outer membrane enables recruitment and activation of Parkin, which promotes the ubiquitination and subsequent lysosomal degradation of many damaged outer membrane proteins (Fig. 13). Parkin recruitment to the mitochondria induces formation of ULK1 that stimulates autophagy.

Reports suggest that stimulation of autophagy/mitophagy in the brains of PD patients may be beneficial for the patients [82–84]. As indicated previously, rapamycin, a widely used autophagy inducer, decreased the levels of protein aggregates (alpha-synuclein) in cellular

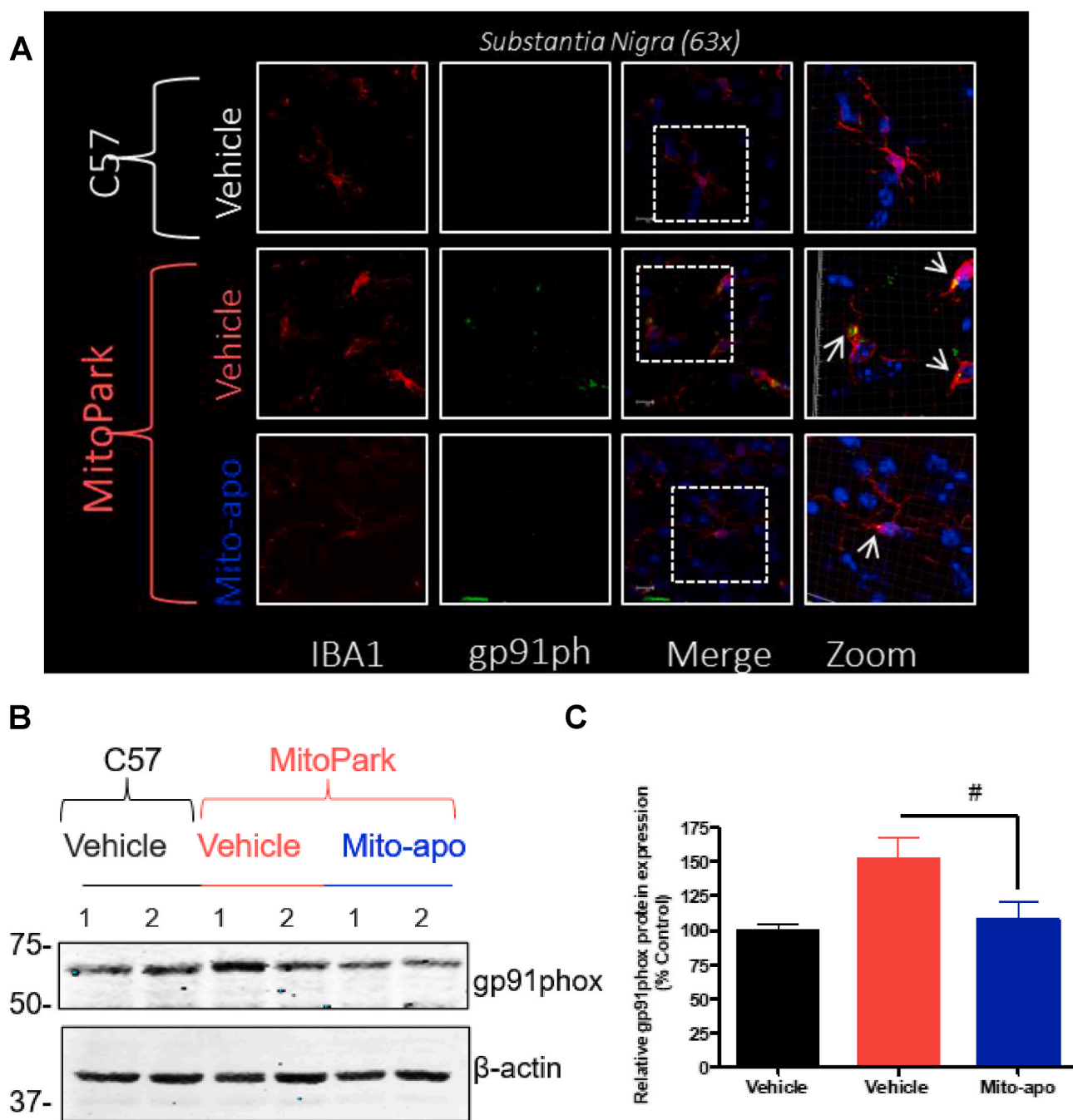


Fig. 15. Mito-Apo inhibits NOX2 expression in the substantia nigra of MitoPark mice. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal.* 2017; 27 (14):1048–1066.

and animal models of alpha-synucleinopathies [85]. Many other studies support the beneficial role of inducers of autophagy in PD, especially in preventing or decreasing the accumulation of alpha-synuclein.

14. PD and neuroinflammation: anti-inflammatory effect of mitochondria-targeted drugs

Neuroinflammation is recognized to play an important role in dopaminergic neurodegeneration in human PD patients and in preclinical mice models [4,19]. Numerous anti-inflammatory agents have been proposed to mitigate neuroinflammation in PD [12]. Ongoing research in our laboratories suggests that mitochondria-targeted drugs could act as anti-neuroinflammatory agents. In cell culture studies, using

macrophages treated with lipopolysaccharide, we observed that several mitochondria-targeted compounds including Mito-Apo could inhibit NOX-mediated superoxide and peroxynitrite (ONOO^-) formation [12, 86,87]. Inhibition of NOX activity mitigates ONOO^- formation in macrophages [86]. In a mouse model of cisplatin-induced nephropathy [88], we had previously shown that mitochondria-targeted nitroxide (Mito-CP) inhibited a second wave of inflammation and mitochondrial damage induced by ROS and reactive nitrogen species (RNS) in the kidney [88]. More importantly, Mito-CP treatment mitigated the expression levels of proinflammatory marker proteins, NOX2 and inducible nitric oxide synthase (iNOS), and oxidative and nitrative damage induced by ONOO^- in the kidney [88].

As described previously, Mito-Apo improved the behavioral and

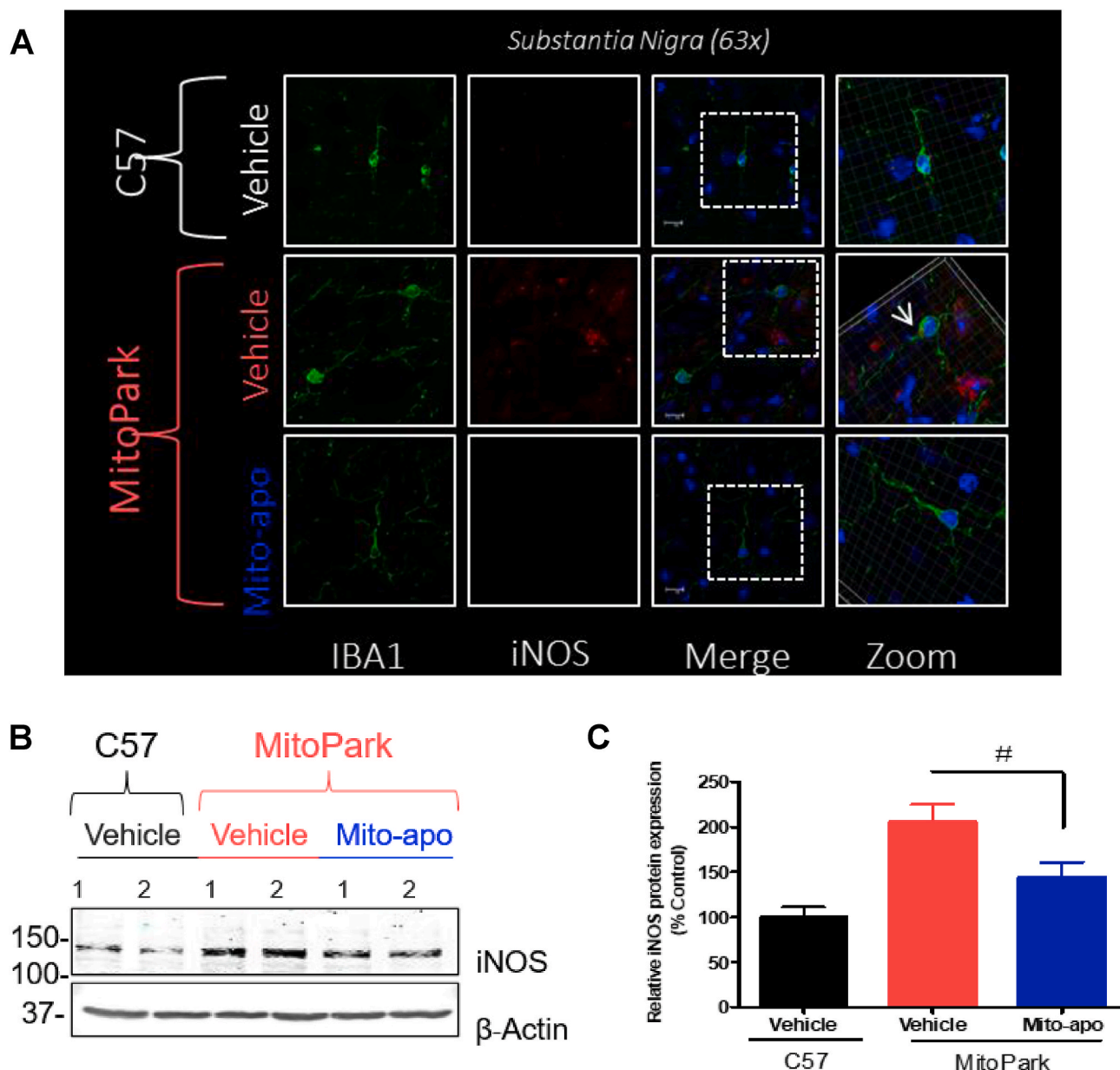


Fig. 16. Mito-Apo inhibits iNOS in the substantia nigra of MitoPark mice. Reprinted from Langley M, Ghosh A, Charli A et al. Mito-Apocynin Prevents Mitochondrial Dysfunction, Microglial Activation, Oxidative Damage, and Progressive Neurodegeneration in MitoPark Transgenic Mice. *Antioxid Redox Signal*. 2017; 27 (14):1048–1066.

neurodegenerative deficits in MitoPark mice [12]. Mito-Apo inhibited microglial activation in the substantia nigra of MitoPark mice (Fig. 14). Previous studies have shown that neuronal cell death occurs from increased generation of potent oxidants from NOX2 and iNOS [12]. Mito-Apo decreased the expression of NOX2 and iNOS in lipopolysaccharide-induced activation of microglia (Figs. 15 and 16, respectively). Mito-Apo inhibited formation of the proinflammatory cytokines in activated microglia. The discovery linking the ability of mitochondria-targeted drugs to inhibit neuroinflammation (Fig. 17) and formation of proinflammatory factors is of great significance in the modulation of inflammation in cancer.

15. Mitochondria-targeted drugs, inflammation, and tumor microenvironment

Reports indicate that mitochondria play a crucial role in modulating immune function [89]. Robust mitochondrial respiration, especially

from mitochondrial complex III, is essential for suppressive function of the regulatory T cells through regulation of DNA methylation status [89]. In animal models of inflammation, mitochondria-targeted compounds were shown to inhibit inflammation that was attributed to their ability to decrease ROS [12,19]. These studies suggest that inflammation can be mitigated through pharmacological intervention targeting mitochondria. In cancer cells, mitochondria-targeting drugs actually enhanced ROS formation in mitochondria through inhibition of mitochondrial complexes and induced the antiproliferative redox signaling mechanism [18,38]. As described in the previous section, mitochondria-targeted drugs conjugated to the TPP⁺ moiety inhibit NOX- and iNOS-generated potent ROS and RNS [12]. This mechanistic scenario may be applicable to proinflammatory events in the tumor microenvironment (TME). The TME consists of regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells (MDSCs). The TME provides a proinflammatory and permissive environment for tumor growth. High levels of ROS/RNS in the TME are

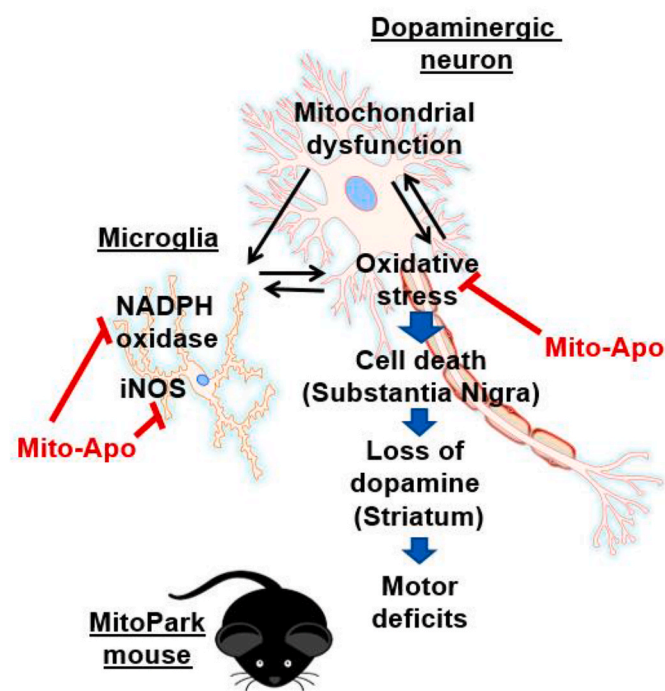


Fig. 17. Anti-inflammatory effects of Mito-Apo for PD therapy.

cytotoxic to T cells, which, unlike cancer cells, lack the antioxidant machinery to combat them and the ability to survive. MDSCs have been shown to inhibit T cell activation through nitration of lymphocyte-specific protein kinase [90]. Clearly, inhibiting ONOO⁻ formation in the TME will convert the proinflammatory TME into an anti-inflammatory TME in which T cells survive and remain activated to destroy tumor cells. As described previously, TPP⁺-containing mitochondria-targeted agents inhibit ONOO⁻ formation and also mitigate the expression of NOX and iNOS levels [12]. New drugs to mitigate inflammation in the TME without causing immunosuppression are highly significant in chemo- and immunotherapies. Ongoing research in our laboratories suggests that mitochondria-targeted drugs inhibit MDSCs and activate T cells in the TME.

16. Summary and future perspectives

PD and cancer share a common pathway linked to mutations in mitochondrial proteins. Increasing evidence supports that targeting mitochondria in both PD and cancer induces neuroprotective and anti-tumor effects. TPP⁺-containing mitochondria-targeting compounds localize in mitochondria, stimulate energy-sensing AMPK activation, and inhibit mTOR signaling via inhibition of mitochondrial respiration and modulation of bioenergetics and mitochondrial biogenesis. Mito-Q was once characterized by its ability to detoxify mitochondrial ROS, but emerging results show that the effects of Mito-Q are complex and multifaceted (e.g., induction of mitophagy). Clearly, additional research, including measurement of toxicity in large animals, pharmacokinetics, and pharmacodynamics, needs to be performed before these compounds can be tested in humans for PD and cancer prevention or treatment, alone or in combination with conventional chemotherapy and radiation therapy. Clearly, the use of mitochondria-targeted drugs in cancer and PD has several limitations and caveats. Mitochondria-targeted drug-mediated inhibition of OXPHOS as a critical mechanism of anti-tumorigenesis is becoming well established in cancer. However, the ability of mitochondria-targeted drugs to afford neuroprotection in diseases like PD, where complex I is already compromised, seems paradoxical. Thus, the neuroprotective ability of mitochondria targeting is dependent on the timing of drug administration and disease progression.

At the early stages of disease onset, these drugs could induce AMPK and mitophagy and enhance neuronal survival. This review is an attempt to teach the basics of mitochondria-targeted drug repurposing from PD to cancer and back to PD using a preclinical animal model.

Declaration of competing interest

The author declares no conflicts of interest.

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References

- [1] J.P. Bernardini, M. Lazarou, G. Dewson, Parkin and mitophagy in cancer, *Oncogene* 36 (10) (2017) 1315–1327.
- [2] S. Matsuda, A. Nakanishi, A. Minami, Y. Wada, Y. Kitagishi, Functions and characteristics of PINK1 and Parkin in cancer, *Front Biosci (Landmark Ed)* 20 (2015) 491–501.
- [3] C. Rotermund, G. Machetanz, J.C. Fitzgerald, The therapeutic potential of metformin in neurodegenerative diseases, *Front. Endocrinol.* 9 (2018) 400.
- [4] M.G. Tansey, M.K. McCoy, T.C. Frank-Cannon, Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention, *Exp. Neurol.* 208 (1) (2007) 1–25.
- [5] M. Naoi, Y. Wu, M. Shamoto-Nagai, W. Maruyama, Mitochondria in neuroprotection by phytochemicals: bioactive polyphenols modulate mitochondrial apoptosis system, function and structure, *Int. J. Mol. Sci.* 20 (10) (2019).
- [6] J. Pan, Y. Lee, G. Cheng, J. Zielonka, Q. Zhang, M. Bajzikova, et al., Mitochondria-targeted honokiol confers a striking inhibitory effect on lung cancer via inhibiting complex I activity, *iScience* 3 (2018) 192–207.
- [7] K. Faust, S. Gehrke, Y. Yang, L. Yang, M.F. Beal, B. Lu, Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease, *BMC Neurosci.* 10 (2009) 109.
- [8] S.E. Seidl, J.A. Potashkin, The promise of neuroprotective agents in Parkinson's disease, *Front. Neurol.* 2 (2011) 68.
- [9] T.M. Ashton, W.G. McKenna, L.A. Kunz-Schughart, G.S. Higgins, Oxidative phosphorylation as an emerging target in cancer therapy, *Clin. Canc. Res.* 24 (11) (2018) 2482–2490.
- [10] M.P. Murphy, R.C. Hartley, Mitochondria as a therapeutic target for common pathologies, *Nat. Rev. Drug Discov.* 17 (12) (2018) 865–886.
- [11] A. Ghosh, K. Chandran, S.V. Kalivendi, J. Joseph, W.E. Antholine, C.J. Hillard, et al., Neuroprotection by a mitochondria-targeted drug in a Parkinson's disease model, *Free Radic. Biol. Med.* 49 (11) (2010) 1674–1684.
- [12] M. Langle, A. Ghosh, A. Charli, S. Sarkar, M. Ay, J. Luo, et al., Mito-apocynin prevents mitochondrial dysfunction, microglial activation, oxidative damage, and progressive neurodegeneration in MitoPark transgenic mice, *Antioxidants Redox Signal.* 27 (14) (2017) 1048–1066.
- [13] T.M. Durcan, E.A. Fon, The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications, *Genes Dev.* 29 (10) (2015) 989–999.
- [14] L.Q. He, J.H. Lu, Z.Y. Yue, Autophagy in ageing and ageing-associated diseases, *Acta Pharmacol. Sin.* 34 (5) (2013) 605–611.
- [15] B. Levine, M. Packer, P. Codogno, Development of autophagy inducers in clinical medicine, *J. Clin. Invest.* 125 (1) (2015) 14–24.
- [16] K.A. Boyle, J. Van Wickle, R.B. Hill, A. Marchese, B. Kalyanaraman, M.B. Dwinell, Mitochondria-targeted drugs stimulate mitophagy and abrogate colon cancer cell proliferation, *J. Biol. Chem.* 293 (38) (2018) 14891–14904.
- [17] F. Weinberg, R. Hamanaka, W.W. Wheaton, S. Weinberg, J. Joseph, M. Lopez, et al., Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity, *Proc. Natl. Acad. Sci. U. S. A.* 107 (19) (2010) 8788–8793.
- [18] G. Cheng, J. Zielonka, O. Ouari, M. Lopez, D. McAllister, K. Boyle, et al., Mitochondria-targeted Analogues of metformin exhibit enhanced antiproliferative and radiosensitizing effects in pancreatic cancer cells, *Canc. Res.* 76 (13) (2016) 3904–3915.
- [19] A. Ghosh, A. Kanthasamy, J. Joseph, V. Anantharam, P. Srivastava, B.P. Dranka, et al., Anti-inflammatory and neuroprotective effects of an orally active apocynin derivative in pre-clinical models of Parkinson's disease, *J. Neuroinflammation* 9 (2012) 241.

- [20] J. Zielonka, J. Joseph, A. Sikora, M. Hardy, O. Ouari, J. Vasquez-Vivar, et al., Mitochondria-targeted triphenylphosphonium-based compounds: Syntheses, mechanisms of action, and therapeutic and diagnostic applications, *Chem. Rev.* 117 (15) (2017) 10043–10120.
- [21] M.P. Murphy, R.A. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations, *Annu. Rev. Pharmacol. Toxicol.* 47 (2007) 629–656.
- [22] J. Asin-Cayuela, A.R. Manas, A.M. James, R.A. Smith, M.P. Murphy, Fine-tuning the hydrophobicity of a mitochondria-targeted antioxidant, *FEBS Lett.* 571 (1–3) (2004) 9–16.
- [23] M.F. Ross, T.A. Prime, I. Abakumova, A.M. James, C.M. Porteous, R.A. Smith, et al., Rapid and extensive uptake and activation of hydrophobic triphenylphosphonium cations within cells, *Biochem. J.* 411 (3) (2008) 633–645.
- [24] J.R. Molina, Y. Sun, M. Protopopova, S. Gera, M. Bandi, C. Bristow, et al., An inhibitor of oxidative phosphorylation exploits cancer vulnerability, *Nat. Med.* 24 (7) (2018) 1036–1046.
- [25] M.F. Beal, Mitochondria take center stage in aging and neurodegeneration, *Ann. Neurol.* 58 (4) (2005) 495–505.
- [26] A.H. Schapira, J.M. Cooper, D. Dexter, P. Jenner, J.B. Clark, C.D. Marsden, Mitochondrial complex I deficiency in Parkinson's disease, *Lancet* 1 (8649) (1989) 1269.
- [27] T.B. Sherer, J.R. Richardson, C.M. Testa, B.B. Seo, A.V. Panov, T. Yagi, et al., Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease, *J. Neurochem.* 100 (6) (2007) 1469–1479.
- [28] J.T. Greenamyre, G. MacKenzie, T.I. Peng, S.E. Stephans, Mitochondrial dysfunction in Parkinson's disease, *Biochem. Soc. Symp.* 66 (1999) 85–97.
- [29] F. Mouton-Liger, M. Jacoupy, J.C. Corvol, O. Corti, PINK1/Parkin-Dependent mitochondrial surveillance: from pleiotropy to Parkinson's disease, *Front. Mol. Neurosci.* 10 (2017) 120.
- [30] E.F. Fang, Y. Hou, K. Palikaras, B.A. Adriaanse, J.S. Kerr, B. Yang, et al., Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease, *Nat. Neurosci.* 22 (3) (2019) 401–412.
- [31] R.L. de Vries, S. Przedborski, Mitophagy and Parkinson's disease: be eaten to stay healthy, *Mol. Cell. Neurosci.* 55 (2013) 37–43.
- [32] E.E. Mowers, M.N. Sharifi, K.F. Macleod, Autophagy in cancer metastasis, *Oncogene* 36 (12) (2017) 1619–1630.
- [33] G. Lim, C. Zhang, K.-L. Lim, Role of autophagy in Parkinson's disease, in: Y. Bailly (Ed.), *Autophagy - A Double-Edged Sword - Cell Survival or Death?*, IntechOpen, London, UK, 2013, pp. 353–375.
- [34] N.D. Georgakopoulos, G. Wells, M. Campanella, The pharmacological regulation of cellular mitophagy, *Nat. Chem. Biol.* 13 (2) (2017) 136–146.
- [35] K. Palikaras, A. Prinz, N. Tavernarakis, Mitophagy modulators, in: *SIS Rattan (Ed.), Encyclopedia of Biomedical Gerontology*, Academic Press, 2020, pp. 433–446, <https://doi.org/10.1016/B978-0-12-801238-3.62137-2>.
- [36] W.H. Koppenol, P.L. Bounds, C.V. Dang, Otto Warburg's contributions to current concepts of cancer metabolism, *Nat. Rev. Cancer.* 11 (5) (2011) 325–337.
- [37] A. Haehner, T. Hummel, H. Reichmann, A clinical approach towards smell loss in Parkinson's disease, *J. Parkinsons Dis.* 4 (2) (2014) 189–195.
- [38] G. Cheng, Q. Zhang, J. Pan, Y. Lee, O. Ouari, M. Hardy, et al., Targeting Ionomycin to mitochondria mitigates lung tumorigenesis and brain metastasis, *Nat. Commun.* 10 (1) (2019) 2205.
- [39] P. Lewis, A. Noyce, The conversation [cited 2019], <https://theconversation.com/parkinsons-four-unusual-signs-you-may-be-at-risk-112035>, 2019.
- [40] M.G. Spillantini, M.L. Schmidt, V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goedert, Alpha-synuclein in Lewy bodies, *Nature* 388 (6645) (1997) 839–840.
- [41] M.F. Beal, Mitochondria and neurodegeneration, *Novartis Found. Symp.* 287 (2007) 183–192, discussion 92–6.
- [42] H. Kumar, H.W. Lim, S.V. More, B.W. Kim, S. Koppula, L.S. Kim, et al., The role of free radicals in the aging brain and Parkinson's Disease: convergence and parallelism, *Int. J. Mol. Sci.* 13 (8) (2012) 10478–10504.
- [43] C. Zhou, Y. Huang, S. Przedborski, Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance, *Ann. N. Y. Acad. Sci.* 1147 (2008) 93–104.
- [44] M. Shamoto-Nagai, W. Maruyama, Y. Hashizume, M. Yoshida, T. Osawa, P. Riederer, et al., In parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity, *J. Neural. Transm.* 114 (12) (2007) 1559–1567.
- [45] The Parkinson Study Group, Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease, *N. Engl. J. Med.* 328 (3) (1993) 176–183.
- [46] A. Martin-Bastida, R.J. Ward, R. Newbould, P. Piccini, D. Sharp, C. Kabba, et al., Brain iron chelation by deferiprone in a phase 2 randomised double-blinded placebo controlled clinical trial in Parkinson's disease, *Sci. Rep.* 7 (1) (2017) 1398.
- [47] A. Dhanasekaran, S. Kotamraju, C. Karunakaran, S.V. Kalivendi, S. Thomas, J. Joseph, et al., Mitochondria superoxide dismutase mimetic inhibits peroxide-induced oxidative damage and apoptosis: role of mitochondrial superoxide, *Free Radical Biol. Med.* 39 (5) (2005) 567–583.
- [48] A. Dhanasekaran, S. Kotamraju, S.V. Kalivendi, T. Matsunaga, T. Shang, A. Keszler, et al., Supplementation of endothelial cells with mitochondria-targeted antioxidants inhibit peroxide-induced mitochondrial iron uptake, oxidative damage, and apoptosis, *J. Biol. Chem.* 279 (36) (2004) 37575–37587.
- [49] M.P. Murphy, Antioxidants as therapies: can we improve on nature? *Free Radic. Biol. Med.* 66 (2014) 20–23.
- [50] R. Betarbet, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov, J. T. Greenamyre, Chronic systemic pesticide exposure reproduces features of Parkinson's disease, *Nat. Neurosci.* 3 (12) (2000) 1301–1306.
- [51] J.W. Langston, P. Ballard, J.W. Tetrud, I. Irwin, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science* 219 (4587) (1983) 979–980.
- [52] R.S. Burns, P.A. LeWitt, M.H. Ebert, H. Pakkenberg, I.J. Kopin, The clinical syndrome of striatal dopamine deficiency. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), *N. Engl. J. Med.* 312 (22) (1985) 1418–1421.
- [53] J. Liu, L.N. Wang, Mitochondrial enhancement for neurodegenerative movement disorders: a systematic review of trials involving creatine, coenzyme Q10, idebenone and mitoquinone, *CNS Drugs* 28 (1) (2014) 63–68.
- [54] R.K. Chaturvedi, M.F. Beal, Mitochondrial approaches for neuroprotection, *Ann. N. Y. Acad. Sci.* 1147 (2008) 395–412.
- [55] G.F. Kelso, C.M. Porteous, C.V. Coulter, G. Hughes, W.K. Porteous, E. C. Ledgerwood, et al., Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties, *J. Biol. Chem.* 276 (7) (2001) 4588–4596.
- [56] B. Kalyanaraman, G. Cheng, M. Hardy, O. Ouari, B. Bennett, J. Zielonka, Teaching the basics of reactive oxygen species and their relevance to cancer biology: mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies, *Redox Biol* 15 (2018) 347–362.
- [57] H.C. Cheng, C.M. Ulane, R.E. Burke, Clinical progression in Parkinson disease and the neurobiology of axons, *Ann. Neurol.* 67 (6) (2010) 715–725.
- [58] J. Stefanski, R. Pawliczak, Apocynin: molecular aptitudes, *Mediat. Inflamm.* 2008 (2008) 106507.
- [59] M.I. Ekstrand, D. Galter, The MitoPark Mouse - an animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons, *Park. Relat. Disord.* 15 (Suppl 3) (2009) S185–S188.
- [60] B.P. Dranka, A. Gifford, D. McAllister, J. Zielonka, J. Joseph, C.L. O'Hara, et al., A novel mitochondrially-targeted apocynin derivative prevents hypoxia and loss of motor function in the leucine-rich repeat kinase 2 (LRRK2(R1441G)) transgenic mouse model of Parkinson's disease, *Neurosci. Lett.* 583 (2014) 159–164.
- [61] Y. Li, W. Liu, T.F. Oo, L. Wang, Y. Tang, V. Jackson-Lewis, et al., Mutant LRRK2 (R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease, *Nat. Neurosci.* 12 (7) (2009) 826–828.
- [62] O. Warburg, The metabolism of carcinoma cells, *J. Canc. Res.* 9 (1) (1925) 148–163.
- [63] O. Warburg, Experiments on surviving carcinoma tissue, *Biochem. Z.* 142 (1923) 317–333.
- [64] O. Warburg, On the origin of cancer cells, *Science* 123 (3191) (1956) 309–314.
- [65] B. Chance, Was Warburg right? Or was it that simple? *Canc. Biol. Ther.* 4 (1) (2005) 125–126.
- [66] G. Cheng, J. Zielonka, B.P. Dranka, D. McAllister, A.C. Mackinnon Jr., J. Joseph, et al., Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death, *Canc. Res.* 72 (10) (2012) 2634–2644.
- [67] N. Sadeghi, J.L. Abbruzzese, S.C. Yeung, M. Hassan, D. Li, Metformin use is associated with better survival of diabetic patients with pancreatic cancer, *Clin. Canc. Res.* 18 (10) (2012) 2905–2912.
- [68] S. Kordes, M.N. Pollak, A.H. Zwinderman, R.A. Mathot, M.J. Weterman, A. Beeker, et al., Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial, *Lancet Oncol.* 16 (7) (2015) 839–847.
- [69] W.K. Miskimins, H.J. Ahn, J.Y. Kim, S. Ryu, Y.S. Jung, J.Y. Choi, Synergistic anticancer effect of phenformin and oxamate, *PLoS One* 9 (1) (2014), e85576.
- [70] J.C. Fitzgerald, A. Zimprich, D.A. Carvajal Berrio, K.M. Schindler, B. Maurer, C. Schulte, et al., Metformin reverses TRAP1 mutation-associated alterations in mitochondrial function in Parkinson's disease, *Brain* 140 (9) (2017) 2444–2459.
- [71] K.G. Lyamzaev, A.V. Tokarchuk, A.A. Panteleeva, A.Y. Mulikidjanian, V. P. Skulachev, B.V. Chernyak, Induction of autophagy by depolarization of mitochondria, *Autophagy* 14 (5) (2018) 921–924.
- [72] T.G. Biel, V.A. Rao, Mitochondrial dysfunction activates lysosomal-dependent mitophagy selectively in cancer cells, *Oncotarget* 9 (1) (2018) 995–1011.
- [73] K. Takeshige, M. Baba, S. Tsuboi, T. Noda, Y. Ohsumi, Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction, *J. Cell Biol.* 119 (2) (1992) 301–311.
- [74] A.M. Leidal, B. Levine, J. Debnath, Autophagy and the cell biology of age-related disease, *Nat. Cell Biol.* 20 (12) (2018) 1338–1348.
- [75] E. Morel, M. Mehrpour, J. Botti, N. Dupont, A. Hamai, A.C. Nascimbeni, et al., Autophagy: a druggable process, *Annu. Rev. Pharmacol. Toxicol.* 57 (2017) 375–398.
- [76] H.C. Yu, C.S. Lin, W.T. Tai, C.Y. Liu, C.W. Shiau, K.F. Chen, Nilotinib induces autophagy in hepatocellular carcinoma through AMPK activation, *J. Biol. Chem.* 288 (25) (2013) 18249–18259.
- [77] S.S. Karuppagounder, S. Brahmachari, Y. Lee, V.L. Dawson, T.M. Dawson, H.S. Ko, The c-Abi inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease, *Sci. Rep.* 4 (2014) 4874.
- [78] J. Bove, M. Martinez-Vicente, M. Vila, Fighting neurodegeneration with rapamycin: mechanistic insights, *Nat. Rev. Neurosci.* 12 (8) (2011) 437–452.
- [79] P. Fagone, M. Donia, K. Mangano, C. Quattrocchi, S. Mamma, M. Coco, et al., Comparative study of rapamycin and temsirolimus demonstrates superimposable anti-tumour potency on prostate cancer cells, *Basic Clin. Pharmacol. Toxicol.* 112 (1) (2013) 63–69.
- [80] J.M.M. Levy, C.G. Towers, A. Thorburn, Targeting autophagy in cancer, *Nat. Rev. Canc.* 17 (9) (2017) 528–542.
- [81] T.N. Nguyen, B.S. Padman, M. Lazarou, Deciphering the molecular signals of PINK1/parkin mitophagy, *Trends Cell Biol.* 26 (10) (2016) 733–744.

- [82] S. Giordano, V. Darley-Usmar, J. Zhang, Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease, *Redox Biol* 2 (2014) 82–90.
- [83] T. Kovács, V. Billes, M. Komlós, B. Hotzi, A. Manzóger, A. Tarnóci, et al., The small molecule AUTEN-99 (autophagy enhancer-99) prevents the progression of neurodegenerative symptoms, *Sci. Rep.* 7 (1) (2017) 42014.
- [84] A. Metaxakis, C. Ploumi, N. Tavernarakis, Autophagy in age-associated neurodegeneration, *Cells* 7 (5) (2018).
- [85] K.A. Malkus, H. Ischiropoulos, Regional deficiencies in chaperone-mediated autophagy underlie alpha-synuclein aggregation and neurodegeneration, *Neurobiol. Dis.* 46 (3) (2012) 732–744.
- [86] J. Zielonka, M. Zielonka, L. VerPlank, G. Cheng, M. Hardy, O. Ouari, et al., Mitigation of NADPH oxidase 2 activity as a strategy to inhibit peroxynitrite formation, *J. Biol. Chem.* 291 (13) (2016) 7029–7044.
- [87] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall, B.A. Freeman, Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide, *Proc. Natl. Acad. Sci. U. S. A.* 87 (4) (1990) 1620–1624.
- [88] A.R. Cardoso, B. Chausse, F.M. da Cunha, L.A. Luevano-Martinez, T.B. Marazzi, P. S. Pessoa, et al., Mitochondrial compartmentalization of redox processes, *Free Radic. Biol. Med.* 52 (11–12) (2012) 2201–2208.
- [89] S.E. Weinberg, B.D. Singer, E.M. Steinert, C.A. Martinez, M.M. Mehta, I. Martinez-Reyes, et al., Mitochondrial complex III is essential for suppressive function of regulatory T cells, *Nature* 565 (7740) (2019) 495–499.
- [90] S. Feng, X. Cheng, L. Zhang, X. Lu, S. Chaudhary, R. Teng, et al., Myeloid-derived suppressor cells inhibit T cell activation through nitrating LCK in mouse cancers, *Proc. Natl. Acad. Sci. U. S. A.* 115 (40) (2018) 10094–10099.