Mitochondrial homeostasis in Parkinson’s disease - a triumvirate rule?

Mitochondrial dysfunction in Parkinson’s disease: Mitochondria are the primary energy generator of the cell and they are important for cell survival and apoptosis. Defective mitochondrial homeostasis is frequently reported in human diseases especially those affecting the brain. Parkinson’s disease (PD), a prevalent neurodegenerative disorder where patients progressively lose control of their movements, is linked to mitochondrial dysfunction. A9-type dopaminergic (DA) neurons in the substantia nigra pars compacta, are significantly diminished in PD brains. These neurons are known to be more susceptible to oxidative stress and mitochondrial toxins compared to other neuronal subtypes. Indeed, exposure to herbicides and pesticides such as paraquat and rotenone are linked to sporadic PD and represents a popular strategy to generate animal models of PD. Furthermore, studies carried out in induced pluripotent stem cells (iPSCs) confirmed the relevance of mitochondrial dysfunction and oxidative stress in PD. Interestingly, familial PD cases are linked to genes with apparent diverse functions that nonetheless converge towards the mitochondrion. These PD-linked genes interact closely with each other through their participations in pathways necessary for the maintenance of mitochondrial homeostasis. For example, a-synuclein (α-syn) and leucine-rich repeat kinase 2 (LRRK2) could regulate mitochondrial fission/fusion. DJ-1, an oxidative stress sensor and protease, localizes to the mitochondria during oxidative stress. The ubiquitin ligase Parkin and mitochondrial serine kinase PTEN-induced putative kinase 1 (PINK1) collaborate to execute mitochondrial quality control through mitophagy, a process whereby damaged mitochondria are removed selectively. The mitochondrial serine protease high temperature requirement A2 ([HtrA2]/Omi) protein acts downstream of PINK1 but in a pathway parallel to Parkin. Interestingly, aberrant mitochondrial fragmentation is often associated with autosomal dominant Parkinsonism caused by mutations in α-syn and LRRK2. On the other hand, autosomal recessive mutations of Parkin, PINK1, DJ-1 and ATPase type 13A2 (ATP13a2) tend to give rise to mitochondrial swelling phenotypes. In essence, we could appreciate the intimate relationship between mitochondrial dysfunction and PD pathogenesis. Given this, unravelling the mechanisms underlying mitochondrial abnormalities holds promise to enhance our understanding of the disease etiology and the development of effective treatment strategies for PD.

AMPK: the chief orchestrator of mitochondrial homeostasis? AMPK-activated protein kinase (AMPK), a serine/threonine kinase, is a well-known intracellular master energy sensor whose activity is tightly linked to the level of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) in the cell. Through this ability, AMPK actively monitors the catabolic and anabolic activity state of the cell. When ATP is abundant, AMPK activity is kept low. When energy is depleted such as during starvation, AMPK is activated by the elevated AMP level, followed by phosphorylation of the enzyme by its upstream kinases such as liver kinase B1 (LKB1), calcium/calmodulin kinase II (CAMKKII) or transforming growth factor beta-activated kinase 1 (TAK1). Although AMPK has many important substrates, our discussion will focus on those linked to mitochondrial homeostasis. At least four known AMPK substrates, mitochondrial fission factor (Mff), dynamin-related protein 1 (Drp1), unc-51-like kinase 1 (Ulk1) and PPARγ coactivator-1a (PGC-1a), are the main executioners of mitochondrial processes such as fission, mitophagy and biogenesis. Activated AMPK could catalyze the phosphorylation of Mff at serine535 and serine172 thereby recruiting Drp1 to activate fission (Toyama et al., 2016). Conversely, the enzyme could inhibit mitochondrial fission by directly phosphorylating Drp1 at serine657. AMPK also regulates autophagy by phosphorylating Ulk1 at serine389 and serine516 or coordinates with protein deacetylase sirtuin 1 (Sirt1) to induce mitochondrial biogenesis by phosphorylating PGC-1a at serine117 and serine166. Furthermore, these processes often crosstalk with each other, for example inducing PGC-1a to suppress mitochondrial fission, and activating fission via Mff/Drp1 is pivotal to mitophagy (Peng et al., 2016; Toyama et al., 2016). As summarized in Figure 1, AMPK is pivotal in coordinating metabolic fate of mitochondria in neurons via a triumvirate rule: modulating mitophagy, fission and biogenesis according to cellular energy requirements. Interestingly, PD gene mutations tend to result in dysregulation of these processes, suggesting that AMPK is a potential therapeutic target for PD. Indeed, pharmacological activators of AMPK such as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and Metformin exhibit some success in improving Parkinsonian phenotypes in experimental and toxin-induced models of PD although conflicting results pointing towards the detrimental effects of AMPK activation were also observed.

Mitochondrial fission is primarily controlled by Drp1, a small cytosolic GTPase. Drp1 translocates to the mitochondria and associates with one of its mitochondrial-bound receptors such as Fis1 or Mff to catalyze membrane separation during fission. Pro-fusion GTPases such as Opalin and Mifusin 1/2 act in an opposite fashion to fission machineries by mediating mitochondrial fusion. AMPK ameliorates mitochondrial fission directly via inhibiting Drp1 activity. Alternatively, AMPK can activate mitochondrial fission during autophagy induction through phosphorylating Mff and recruitment of Drp1 (Toyama et al., 2016). Thus, AMPK plays a Janus-like role in regulating mitochondrial fission. Both fission and fusion play equally important roles in neurons in terms of mitochondrial homeostasis and ATP synthesis. Mitochondrial fission is critical for mitophagy and also for mitochondrial motility. As smaller mitochondria are more mobile, mitochondrial fission ensures that the organelle are of the appropriate size to be trafficked along the axons. Indeed, loss of Drp1 in DA neurons leads to axonal degeneration as a result of the reduction in mitochondrial mass at the synapses due to impaired trafficking of mitochondria (Berthet et al., 2014). Mifusin 1 and 2 are targets of PARK1/Parkin-dependent ubiquitination during mitophagy, probably as a way to fuse damaged mitochondria to endoplasmic reticulum (ER) or lysosome for degradation (Gegg et al., 2010). Pharmacologically and genetically inhibiting Drp1 has been shown to prevent dopaminergic neuronal loss and restore dopamine release in PARK1-deficient mice. Familial LRRK2 point mutation G2019S increased Drp1 phosphorylation at thre-
onine resulting in hyperfission and augmented autophagy (Su et al., 2013). Inhibiting Drp1 activity using mimetic peptide or dominant negative Drp1 mutant could reverse mitochondrial dysfunction and protect against degeneration of DA neurons derived from iPSCs from this patient. Overall, inhibiting fission or ameliorating augmented autophagy seems to be rather promising approaches against certain forms of PD.

PGC-1α is important for the survival of DA neurons. Conditional knockout of PGC-1α leads to the reduction in DA neuron (Jiang et al., 2016). We have reported similar observation in Drosophila spargel mutant, an ortholog to mammalian PGC-1α (Ng et al., 2017). These spargel mutants exhibit marked reduction in PGC-1α mRNA transcripts, exhibit age-dependent loss of mobility and significant depletion of dopamine due to degeneration of DA neurons. Interestingly, over-expression PGC-1α could also modulate the protein level as well as the activity of fission/fusion proteins Drp1 and Mfn2 in PC12 cells treated with rotenone, which suggests that a crosstalk between mitochondrial biogenesis and fission/fusion machineries (Peng et al., 2016).

Defective mitochondrial biogenesis in PD: PGC-1α is a master regulator of mitochondrial biogenesis. Although the role of PGC-1α in skeletal muscle autophagy and mitochondrial biogenesis had been well studied, far less was done in term of understanding its roles in neurons. PGC-1α has been reported to be remarkably downregulated in PD patients, which implies a relationship between mitochondrial biogenesis and PD. Conditionally knocking out PGC-1α in adult mousebrain results in the loss of dopaminergic neurons (Jiang et al., 2016). A study done using Drosophila PGC-1a homolog spargel yields similar conclusion (Ng et al., 2017).

A novel relationship between Parkin and PGC-1α was first reported by Shin et al. in 2011. In this report, the investigators discover Parkin’s role in regulating PGC-1α transcription. Parkin ubiquitinates Parkin interacting substrate (PARIS/ZNF746), a repressor of PGC-1α, targeting it for proteasomal degradation. In the absence of Parkin, PARIS is stabilized and bound to the promoter of PGC-1α, switching off PGC-1α expression that results in DA neuronal death (Figure 2).

Three separate studies also further established that transcriptional repression of PGC-1α as a mechanism underlying neurotoxin effects and neuroinflammation in PD. Siddiqui et al. in 2012 reported that mitochondrial toxin manipulates mitochondrial biogenesis in 2 ways, increasing α-syn-dependent repression as well as decreasing myocyte enhancer factor 2C (MEF2C)-mediated transcription of PGC-1α, with oxidative stress exerting a synergistic effect. Oxidative stress enhances α-syn-dependent repression and S-nitrosylation of MEF2C leading to decreased biogenesis. Furthermore, the investigators observed that mutant α-syn A53T is twice as efficient in repressing the promoter of PGC-1α compared to wild-type α-syn. This conclusion was also supported by another study whereby the investigators observed DA neurons in PGC1α-KO mice show abnormal mitochondria and fragmented endoplasmic reticulum and further expression of human α-syn exacerbated degeneration of these vulnerable neurons (Ciron et al., 2015).

To understand the impact of neuroinflammation, a prominent feature of PD, the pro-inflammatory fatty acid, palmitate was administered to ex vivo cultured primary mouse cortical neurons, microglia and astrocytes. Palmitate-treated cells showed significant cytosine methylation in PGC-1α promoter resulting in reduced mitochondrial content (Su et al., 2015). Similarly intracerebroventricular injection of palmitate into mice overexpressing human α-syn resulted in enhanced methylation of PGC-1α promoter, decreased PGC-1α expression and reduced mitochondrial content in substantia nigra.

Last but not least, the level of PGC-1α was significantly decreased in mnd2 (motor neuron degeneration 2) mouse, a mutant mouse strain harboring homozygous recessive mutation in Omi (Xu et al., 2014). Mnd2 mice exhibit neurodegeneration, mitochondrial abnormalities, stunted growth and rarely survived beyond 40 days postnatally. Levels of NRF-1 and TFAM mRNA which are targets of PGC-1α, were found to be markedly reduced in mnd2 mouse brain compared with wide-type suggesting that Omi may be associated with the reduction of PGC-1α. Omi was found to cleave glycogen synthase kinase 3β (GSK3β), and the latter can phosphorylate PGC-1α and promote its ubiquitin-mediated degradation.

Conclusions: We have discussed here the participation of several mitochondrial pathways such as fission, mitophagy and biogenesis in the pathogenesis of PD. Given that seemingly diverse genes associated with PD all converged at the mitochondria, one could envisage the possibility to counteract neurodegeneration, especially in the case of PD, via mitochondrial-related therapeutics. In so far, most results suggest that the mitochondrial pathology in PD may be due to the imbalance of the mitochondrial dynamics or biogenesis, the latter clearly illustrates the importance of PGC-1α in DA neuronal survival. Notably, two animal studies, one in rodent and the other in Drosophila, robustly support the importance of PGC-1α in DA neuronal survival. Transcriptional repression of PGC-1α promoter is another important pathological mechanism that exemplifies the role of PGC-1α in PD. Further investigations are needed to see if a common pathway such as those regulated by AMPK or its substrates could be tapped for treating PD.

Chee-Hoe Ng, Liting Hang, Kah-Leong Lim
National Neuroscience Institute, Singapore (Ng CH, Hang L, Lim KL)
Neuroscience & Behavioral Disorders Program, Duke-NUS Medical School, Singapore (Lim KL)
Department of Physiology, National University of Singapore, Singapore (Lim KL)
NUS Graduate School for Integrative Sciences and Engineering, Singapore (Hang L)

*Correspondence to: Chee-Hoe Ng, Ph.D., CheeHoe267@yahoo.com.sg.
orcid: 0000-0001-8545-0497 (Chee-Hoe Ng)
0000-0002-5440-2588 (Kah-Leong Lim)
Accepted: 2017-07-24

doi: 10.4103/1673-5374.213546

How to cite this article: Ng CH, Hang L, Lim KL (2017) Mitochondrial homeostasis in Parkinson’s disease - a triumvirate rule? Neural Regen Res 12(8):1270-1272.

Plagiarism check: Checked twice by iThenticate.
Peer review: Externally peer reviewed.
Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon
Figure 1 PD genes interact with mitochondrial fission, biogenesis and mitophagy.
AMPK is a master energy sensor that is activated by increased cellular AMP to ATP ratio. It participates in mitochondrial homeostasis by phosphorylating some key regulators such as Drp1, Mff, Ulk1, Raptor and PGC-1α. When AMP accumulates such as during starvation, mitophagy is favored and AMPK activates this mechanism via coordinating phosphorylation of Raptor and Ulk1. Alternatively, AMPK activates autophagy through an intermediate step that requires mitochondrial fission via phosphorylating Mff and recruitment of Drp1 (Toyama et al., 2016). In addition, AMPK together with Sirt1 (not shown here), participates in post-transcriptional modification of PGC1α to re-establish mitochondrial population through biogenesis. AMPK can oppose mitochondrial fission directly via inhibiting Drp1 activity. Blocking fission could be a means to elevate cellular ATP. PGC-1α over-expression can result in inhibition of fission and enhanced fusion. Mutations in PD genes such as α-syn, ATP13a2, LRRK2, Parkin, PINK1 disrupt mitochondrial homeostasis along these pathways. Parkin may polyubiquitinate Mff and this targets damaged mitochondria for clearance. Mutations of DJ-1 or α-syn enhanced mitochondrial fragmentation by inducing Drp1 expression or activation, respectively. Neurons which are impaired in mitophagy may accumulate dysfunctional mitochondria and unable to adapt to stress while those inadequate in biogenesis will not be able to cope with energy demand. α-syn: α-Synuclein; AMP: adenosine monophosphate; AMPK: AMP-activated protein kinase; ATP: adenosine triphosphate; ATP13a2: ATPase type 13A2; Drp1: dynamin-related protein 1; LRRK2: leucine-rich repeat kinase 2; Mff: mitochondrial fusion factor; PD: Parkinson’s disease; PGC-1α: PPARγ coactivator-1 α; PINK1: PTEN-induced putative kinase 1; Sirt1: sirtuin 1; Ulk1: unc-51-like kinase 1.

Figure 2 Transcriptional repression of PGC-1α by PD genes, mitochondrial insults and neuroinflammation.
Nuclear α-syn can bind directly to PGC-1α promoter, resulting in repression of PGC-1α transcription (Siddiqui et al., 2012). α-syn AS3T and mitochondrial toxin (MToxin) can induce N-nitrosylation of transcription factor MEF2C, inhibiting transcriptional activation of PGC-1α (Ryan, 2013). Parkin and Omi, target PARIS and GSK3β for degrada- tion, respectively (Shin et al., 2011; Xu et al., 2014). PARIS acts as a repressor of PGC-1α promoter, while GSK3β promotes PGC-1α degradation. Finally, palmitate, a pro-inflammatory fatty acid, could elicit epigenetic modification by methylating cytosine in PGC-1α promoter in primary cortical neurons, astrocytes and microglial cells (Su et al., 2015). This modification represses PGC-1α expression, giving rise to reduce mitochondrial biogenesis and aberrant fission/fusion. It remains unclear if any of these have impact on autophagy: α-syn: α-Synuclein; GSK3β: glycogen synthase kinase 3β; MEF2C: myocyte enhancer factor 2C; PARIS: Parkin interacting substrate; PD: Parkinson’s disease; PGC-1α: PPARα coactivator-1 α.

Ng CH, Basal AH, Hang L, Tan R, Goh KL, O’Neill S, Zhang X, Yu F, Lim KL (2017) Genetic or pharmacological activation of the Drosophila PGC-1α orthologuspargel rescues the disease phenotypes of genetic models of Parkinson’s disease. Neurobiol Aging 55:33–37.

Peng K, Yang L, Wang J, Ye F, Dan G, Zhao Y, Cai Y, Cui Z, Ao L, Liu J, Zou Z, Sai Y, Cao J (2016) The interaction of mitochondrial biogenesis and fusion/fission mediated by PGC-1α regulates rotone-induced dopaminergic neurotoxicity. Mol Neurobiol 54:373-379.

Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M, Lee B, Lopez K, Nutter A, Shan B, Molokanova E, Zhang Y, Han X, Nakamura T, Masilah E, Yates JR 3rd, et al. (2013) Isogenic human iPSC Parkinson’s model shows nitrosative stress-induced dysfunction in MEF2-PGC1α transcription. Cell 155:1351-1366.

Shin JH, Ko HS, Kang H, Lee Y, Lee YJ, Petikova O, Troconzo JC, Dawson VL, Dawson TM (2011) PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson’s disease. Cell 144:689-702.

Siddiqui A, Chinta SJ, Mallajosyula JK, Rajagopalan S, Hanson I, Rane A, Melov S, Andersen JK (2012) Selective binding of nuclear alpha-synuclein to the PGC1α promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson’s disease. Free Radic Biol Med 53:993-1003.

Su X, Chu Y, Kordower JH, Li B, Cao H, Huang L, Nishida M, Song L, Wang D, Federoff HJ (2015) PGC-1α promoter methylation in Parkinson’s disease. PLoS One 10:e0134087.

Su Y, Qi X (2013) Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by LRRK2 G2019S mutation. Human Mol Genet 22:4545-4561.

Toyama EK, Herzig S, Courchet J, Lewis TL Jr, Losón OC, Hellberg K, Young S, Federoff HJ (2015) PGC-1α: PPARα coactivator-1 α.

References
Berthet A, Margolis EB, Zhang J, Hsieh I, Zhang J, Hnasko TS, Ahmad J, Edwards RH, Sesaki H, Huang EL, Nakamura K (2014) Loss of mitochondrial fission depletes axonal mitochondria in midbrain dopamine neurons. J Neurosci 34:14304-14317.

Ciron C, Zheng L, Bohlen W, Knott GW, Kelly DP, Schneider BL (2015) PGC-1α activity in nigral dopamine neurons determines vulnerability to α-synuclein. Acta Neuropathol Commun 3:16.

Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Tainman JW (2010) Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Hum Mol Genet 19:4861–4870.

Jiang H, Kang SU, Zhang S, Karuppagounder S, Xu J, Lee YK, Kang BG, Lee Y, Zhuang J, Petikova O, Troconzo JC, Prowoza S, Andradi SA, Dawson VL, Dawson TM (2016) Adult conditional knockout of PGC-1α leads to loss of dopamine neurons. eNeuro 3:ENEURO.0183-16.2016.