

● PERSPECTIVE

Mitochondria in the context of Parkinson's disease

Introduction to the disease: Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 1–2% of the population over the age of 65. Rigidity, bradykinesia, postural instability and tremor are the principal clinical characteristics of this disease, caused by the progressive loss and degeneration of dopaminergic neurons in the *Substantia nigra pars compacta* (SNpc). Tremor and motor impairment appear when approximately 50–60% of these neurons degenerate, causing a 70–80% depletion of striatal dopamine (Lang and Lozano, 1998). The pathogenesis of PD involves a series of characteristics that are commonly observed in the affected brain areas of PD patients, like mitochondrial complex I and global lysosomal dysfunctions. Other features of the disease are increased indices of oxidative stress linked to a dysregulated inflammatory response or the presence of intra-neuronal protein aggregates designated as Lewy bodies (LBs), composed principally of metabolically altered alpha-synuclein. This dopaminergic neurodegeneration is closely related to autophagic degradation and protein aggregation, two especially important processes in the viability of the neuron (Ryan et al., 2015).

Relation between neuron degeneration and mitochondrial activity: Mitochondria are deeply dynamic and complex organelles that constantly divide and fuse with each other, move along the cell, suffer regulated turnover and play diverse important cellular functions. The adequate balance between fusion and fission at the appropriate time and subcellular location is crucial for the maintenance of mitochondrial activity and several cellular requirements. Abnormalities in this balance can lead to neuron dysfunction and cell death. In these terms, a selective autophagic degradation of mitochondria, named mitophagy, is necessary for the turnover or removal of damaged mitochondria (Wang et al., 2011). Mitophagy eliminates dysfunctional mitochondria and avoids the production of excessive reactive oxygen species (ROS) caused by mitochondrial oxidative respiration.

Neurons are especially energy-dependent cells and in the case of dopaminergic neurons, this dependence is even higher. Dopamine is a quite unstable neurotransmitter that can oxidize to generate reactive oxygen species and produce neurotoxicity (Liang et al., 2007). Therefore, neurons are extremely sensitive to mitochondrial injury or to anomalous distribution, which can result in severe consequences for neuronal function and survival. SNpc dopamine (DA) neurons are even more vulnerable to PD effects than dopamine neurons of ventral tegmental area (VTA) or interfascicular (IF) area. This selective vulnerability of neuronal sub-populations is still being subject of study and possibly the cause of this selectivity is a combination of different phenomena. A high amount of iron ions and low levels of the antioxidant glutathione in SNpc DA cells have been described as possible causes. Calcium toxicity has been also suggested as another determinant of vulnerability. SNpc DA neurons present more calcium channels than VTA DA neurons, which have a preponderance of sodium channels, and less level of calcium-binding proteins. On the other hand, the levels of VMAT2, the vesicular monoamine transporter responsible for dopamine uptake, are lower in SNpc DA neurons. However, other possible explanation that can also contribute to the selective vulnerability is the reduced mitochondria mass presented in SNpc DA neurons compared to VTA or IF DA neurons (Liang et al., 2007). The low mitochondrial mass predisposes these neurons to degeneration when mitochondria function is impaired.

Anomalous mitochondrial dynamics (mitophagy included) represent a key common pathway during the course of neurodegenerative diseases. Numerous pieces of evidence suggest that mitochondrial dysfunction is a central and critical player in the pathogenesis of various neurodegenerative diseases, and an aberrant mitochondrial homeostasis is supposed to be an essential contributor to the development of PD (Wang et al., 2011). This evidence was first collected in the early 1980s with the observation that a potent inhibitor of com-

plex I of the electron transport chain, MPTP, rapidly induced Parkinsonism (Langston et al., 1983). Although the cause of mitochondrial dysfunction in PD is not entirely clear, sporadic and familial PD seem to converge at the level of mitochondrial integrity. Moreover, the role of various familial PD-associated genes in the regulation of mitochondrial function and dynamics give as a clue to start elucidating the connections of mitochondria with PD (Winklhofer and Haass, 2010).

Molecular mechanisms in PD: Although the majority of PD cases are sporadic (around 90%), autosomal dominant and recessive familial cases have been linked to mutations in the genes for alpha-synuclein, Parkin, PINK1, DJ1 or LRRK2. Both sporadic and familial forms of PD share important clinical, pathological and biochemical characteristics, being the progressive damage of dopaminergic neurons in the substantia nigra especially noteworthy. Notwithstanding the prevalence of sporadic PD cases, the familial forms can provide exceptional insights into the molecular mechanism of disease pathogenesis (Winklhofer and Haass, 2010).

PARK2 is one of the most common genes mutated in cases of early-onset familial PD, and its mutations are associated with mitochondrial dysfunction. *PARK2* encodes an E3 ubiquitin ligase, Parkin, which can be selectively translocated to damaged mitochondria to promote their removal by autophagy (Pickrell et al., 2015). Recessive loss-of-function mutations are responsible for disrupting its ubiquitin ligase activity necessary to target dysfunctional mitochondria for selective mitophagy. Parkin normally works with PINK1 (the other most common causal gene for recessive familial parkinsonism, *PARK6*) in the same pathway to control mitochondrial quality and elimination of damaged mitochondria.

There are other PD-related proteins that have a link to mitochondria, apart from Parkin and PINK1. In fact, one of the main features of the disease is the presence of intra-cytoplasmic inclusions containing alpha-synuclein protein (called Lewy bodies), typically found in areas of neuronal degeneration. Alpha-synuclein also accumulates in the mitochondria of the striatum and substantia nigra of PD patients, impairing this way mitochondrial activity and causing oxidative stress. Moreover, mutant alpha-synuclein overexpression induces mitophagy and the loss of Parkin aggravates this phenotype, suggesting that synuclein pathology may meet the mitophagy-related PINK1/Parkin pathway (Chen et al., 2015). Analyses of potential mechanistic links between these and other PD disease genes are active areas of investigation (Okatsu et al., 2012; Pickrell et al., 2015).

It is also known that in damaged mitochondria, the loss of mitochondrial membrane potential ($\Delta\Psi_m$) results in the import of protein PINK1 to the outer mitochondrial membrane via its mitochondrial-targeting signal (MTS) and remains associated with the TOM complex (translocase of the outer membrane). Dissipation of mitochondrial membrane potential prevents PINK1 from reaching the inner membrane, causing its autophosphorylation and the accumulation of non-cleaved PINK1 on damaged mitochondria (Okatsu et al., 2012). At the same time, PINK1 directly phosphorylates ubiquitin at Ser65 and Parkin at Ser65 within its Ubl domain, leading to the activation of the E3 ligase activity of Parkin and its retention on depolarized mitochondria (Pickrell et al., 2015). Recruited Parkin builds ubiquitin chains on outer membrane proteins, preferentially on which PINK1 has accumulated, notably the mitofusins. Ubiquitylation of outer mitochondrial membrane proteins by Parkin leads either to their degradation by the proteasome or to the removal of the damaged mitochondria by autophagy. Patient mutations in both PINK1 and Parkin prevent PINK1 autophosphorylation and recruitment of Parkin to mitochondria (Okatsu et al., 2012). PINK1 and Parkin dependent ubiquitylation has a main role in the quality control of mitochondria, which suggests that the dysfunction of either PINK1 or Parkin results in the accumulation of low quality mitochondria and consequent cell damage and neuronal dysfunction, contributing this way to the development of PD. Organellar degradation system, in which Parkin is involved, is fundamental to several different cellular events and its malfunction is thought to be a responsible key to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis. The absence of functional Parkin is thought to result in aberrant ubiquitylation and compromised mitochondrial integrity, leading

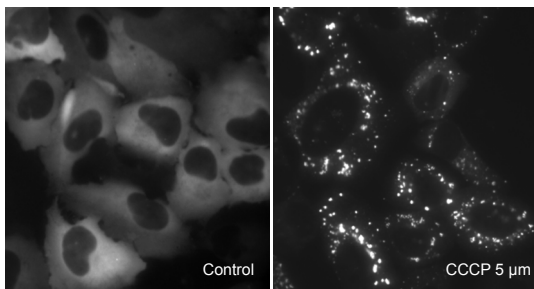


Figure 1 Fluorescent Parkin-based preclinical model.

After the addition of the mitochondrial membrane uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) to the culture, the fluorescently tagged and stably expressed Parkin is recruited to the damaged mitochondria. The difference in the cellular fluorescence redistribution is highly appreciable; from a cytoplasmic distribution in the negative control, it turns into a vesicular pattern after CCCP treatment, corresponding to mitochondrial distribution.

to cell damage and neuronal dysfunction, contributing to the development of Parkinson's disease. Nevertheless, the exact mechanism of mitochondrial pathogenesis associated with Parkinson's disease and Parkin protein in particular has not been totally elucidated (Villacé et al., 2016).

The future: searching for mitochondrial therapeutic targets: Therapies for the disease are limited and treatments to date only provide symptomatic relief without stopping disease progression. While neuroregeneration is conceptually distinct from neuroprotection, from the perspective of therapeutic development in PD, any effect that decelerates or stops the course of neurodegeneration will be welcomed. Any mechanism by which an agent may protect against neuronal degeneration, might also allow endogenous repair processes, therefore it is no necessary distinguish between these concepts. However, preclinical studies should be performed on relevant disease models that best reflect the pathologic condition. This relevance is one of the main reasons that explain the low rate of success of drug development in neuroscience. For example, supplementation with mitochondrial complex I electron acceptor coenzyme Q₁₀ has been demonstrated to be beneficial in various preclinical models of Parkinson's disease, but without clinical benefits in Phase 3 trials (Ryan et al., 2015). More translational assays would eventually help saving time and efforts by increasing the precision to identify reliable hits. The regulation of targets involved in this pathway has been a tricky area for drug discovery and drug development, basically because of a lack of good robust and functional assays for screening. It is of the utmost interest to develop accurate methods to understand the specific function of Parkin in mitochondria in the context of PD.

Recent researches on the function of PD-associated genes have provided fundamental new insights into biochemical pathways of the disease process. As commented before, some of these genes are directly implicated in mitochondrial dynamic (Winklhofer and Haass, 2010). Being mitochondrial dysfunction a common denominator of sporadic and familial PD, mitochondria has moved to the front of PD research. Many sides of mitochondrial biology are affected in PD. Explaining and interpreting the functions of the proteins implicated in familial forms of PD has become a valid strategy to understand mitochondrial cell biology and its connection with and contribution to sporadic disease. For example, the ATP analog, kinetin triphosphate, or its precursor kinetin, rescues kinase activity of patient mutant form of PINK1 and increases wild type PINK1 activity, resulting in increased parkin recruitment to damaged mitochondria, decreasing sensitivity to oxidative stress and revealing a new kind of drug in the treatment of PD (Hertz et al., 2013). So, the activation of mitophagy by regulating PINK1 or Parkin pathway is becoming an attractive target not only for therapeutic intervention for PD, but also for the attempt of treating any mitochondrial diseases. Deeply understanding the participation of mitochondrial dysfunction in neurodegeneration will necessarily enrich our capacity to develop more adequate therapies for neurodegenerative diseases (Villacé et al., 2016).

In the process of discovering new drugs, High Content Cell-based assays are becoming a very important tool to simulate the nature of biological processes and essential for the identification and optimization of therapeutic candidates. Advances in High Content Screening (HCS) technologies will contribute to increase the efficiency in drug discovery process. Some improvements related to this area have been performed recently. Our lab has developed, for instance, a High-Content Assay based on a phenotypic Parkin cell-based model (Figure 1) that permits to evaluate robustly the fluorescently tagged Parkin mitochondrial recruitment in living cells through the study of its location pattern in the space and time during the early stages of mitophagy, after promoting a loss of mitochondrial membrane potential ($\Delta\Psi_m$) with a mitochondrial proton gradient uncoupler agent (Villacé et al., 2016). Different screenings assays have been carried out as well by means of screening *ex vivo* PD patients' cells. For example, a library screening completed with fibroblasts from patients carrying parkin mutations identified 15 compounds that normalized MMP and ATP levels (Mortiboys et al., 2013) in these cells. Following this line of experimentation, next generations of High-Throughput or High-Content Screenings using patients' cells or iPSC neuronal cultures should represent a very promising strategy for assessing preclinical efficacy and safety on individually targeted therapies (Ryan et al., 2015). Finding activators and inhibitors of the PINK1/Parkin pathway and others will accelerate the discovery of achievable candidates against Parkinson's disease.

Patricia Villacé, Rosa M. Mella, Danel Kortazar*
InnoproT (Innovative Technologies in Biological Systems), Derio, Spain
*Correspondence to: Danel Kortazar, Ph.D., dkortazar@innoproT.com.

Accepted: 2017-02-15

orcid: 0000-0002-2871-8124 (Danel Kortazar)

doi: 10.4103/1673-5374.200802

How to cite this article: Villacé P, Mella RM, Kortazar D (2017) Mitochondria in the context of Parkinson's disease. *Neural Regen Res* 12(2):214-215.

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 the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

References

- Chen L, Xie Z, Turkson S, Zhuang X (2015) A53T human alpha-synuclein overexpression in transgenic mice induces pervasive mitochondria macroautophagy defects preceding dopamine neuron degeneration. *J Neurosci* 35:890-905.
- Hertz NT, Berthet A, Sos ML, Thorn KS, Burlingame AL, Nakamura K, Shokat KM (2013) A neo-substrate that amplifies catalytic activity of parkinson's-disease-related kinase PINK1. *Cell* 154:737-747.
- Lang AE, Lozano AM (1998) Parkinson's disease. First of two parts. *N Engl J Med* 339:1044-1053.
- Langston JW, Ballard P, tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219:979-980.
- Liang CL, Wang TT, Luby-Phelps K, German DC (2007) Mitochondria mass is low in mouse substantia nigra dopamine neurons: Implications for Parkinson's disease. *Exp Neurol* 203:370-380.
- Mortiboys H, Aasly J, Bandmann O (2013) Ursocholic acid rescues mitochondrial function in common forms of familial Parkinson's disease. *Brain* 136:3038-3050.
- Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, Tani N, Kimura M, Go E, Koyano F, Funayama M, Shiba-Fukushima K, Sato S, Shimizu H, Fukunaga Y, Taniguchi H, Komatsu M, Hattori N, Mihara K, Tanaka K, Matsuda N (2012) PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nat Commun* 3:1016.
- Pickrell AM, Huang C, Kennedy SR, Ordureau A, Sideris DP, Hoekstra JG, Harper JW, Youle RJ (2015) Endogenous Parkin preserves dopaminergic substantia nigral neurons following mitochondrial DNA mutagenic stress. *Neuron* 87:371-381.
- Ryan BJ, Hoek S, Fon EA, Wade-Martins R (2015) Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends Biochem Sci* 40:200-210.
- Villacé P, Mella RM, Roura-Ferrer M, Valcárcel M, Salado C, Castilla A, Kortazar D (2016) Fluorescent parkin cell-based assay development for the screening of drugs against Parkinson's disease. *J Biomol Screen* doi:10.1177/1087057116671498.
- Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL (2011) PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 147:893-906.
- Winklhofer KF, Haass C (2010) Mitochondrial dysfunction in Parkinson's disease. *Biochim Biophys Acta* 1802:29-44.