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Review

Mitophagy and Parkinson's disease: Be eaten to stay healthy

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ABSTRACT

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders. Pathologically, it is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Although most occurrences have an unknown cause, several gene mutations have been linked to familial forms of PD. The discovery of some of the proteins encoded by these genes, including Parkin, PINK1 and DJ-1, at the mitochondria offered a new perspective on the involvement of mitochondria in PD. Specifically, these proteins are thought to be involved in the maintenance of a healthy pool of mitochondria by regulating their turnover by mitochondrial autophagy, or mitophagy. In this review, we discuss recent studies on the role of mitophagy in PD. We present three putative models whereby PINK1 and Parkin may affect mitophagy; 1) by shifting the balance between fusion and fission of the mitochondrial network, 2) by modulating mitochondrial motility and 3) by directly recruiting the autophagic machinery to damaged mitochondria. This article is part of a Special Issue entitled 'Mitochondrial function and dysfunction in neurodegeneration'.

Contents

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's Disease, and affects over one million people in the United States (Dauer and Przedborski, 2003). Clinically, it is characterized by resting tremor, bradykinesia, rigidity and postural instability (Fahn and Przedborski, 2005), which are primarily caused by a profound deficit in brain dopamine. In addition, dementia and depression are common (Dauer and Przedborski, 2003). The most prominent pathological features of PD are the loss of pigmentation in the substantia nigra pars compacta (SNc) – corresponding to loss of neuromelanin-containing dopaminergic neurons in the ventral midbrain – and the presence of intra-cytoplasmic proteinaceous inclusions called Lewy bodies (Gasser, 2009), which consist mainly of α-synuclein. Especially in advanced disease, this neurodegeneration extends well beyond dopaminergic neurons and is also found in the noradrenergic, serotonergic and cholinergic systems.

PD occurs mostly sporadically and its cause remains unknown. Research in PD has long concentrated on alterations in the nigrostriatal pathway, including defects in mitochondrial bioenergetics (Schon and Przedborski, 2011). The discovery of mutations in the genes encoding Parkin (PARK2) (Kitada et al., 1998), PINK1 (PARK6) (Valente et al., 2004) and DJ-1 (PARK7) (Bonifati et al., 2003), all proteins associated with the mitochondria, raised questions about a role for mitochondrial

Abbreviations: (PD), Parkinson's Disease; (SNc), substantia nigra pars compacta; (PINK1), phosphatase and tensin homolog-induced putative kinase 1; (MOM), mitochondrial outer membrane; (CCCP), carbonyl cyanide m-chlorophenylhydrazone; ($\Delta\Psi_m$), mitochondrial membrane potential; (mtDNA), mitochondrial DNA.

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quality control in PD. Although mitochondria are often depicted in textbooks as singular oval shaped organelles, in reality they form dynamic networks that constantly undergo fusion and fission (Chen and Chan, 2009). Beyond production of ATP, mitochondria are involved in regulation of a host of other processes such as calcium homeostasis and apoptosis (Newmeyer and Ferguson-Miller, 2003). They are constantly under threat of being damaged by reactive oxygen species (ROS) and it is thought that the process of mitochondrial autophagy, or mitophagy, is important to maintain an overall healthy population (Twig et al., 2008a,b). When this fails, damaged mitochondria may accumulate in the cell. Especially in neurons, which are post-mitotic and rely heavily on energy production by mitochondria, an accumulation of damaged mitochondria may be harmful and might lead to neuronal cell death (Chen and Chan, 2009). Indeed, several studies have found an increase in mitochondrial DNA (mtDNA) mutations and mitochondrial dysfunction in SNc neurons of PD patients compared to age-matched controls (Bender et al., 2006; Mann et al., 1994; Schapira et al., 1990).

Below, we will discuss recent studies on the role of mitophagy in PD. Although DJ-1 and, recently, α-synuclein have also been implicated in this process, the majority of research has been dedicated to PINK1 and Parkin and these proteins will be the focus of this review.

PINK1 and Parkin

Mutations in the genes encoding phosphatase and tensin homolog-induced putative kinase 1 (PINK1) and Parkin lead to recessive, early onset forms of PD (Nuytemans et al., 2010). These proteins were first suggested to share a pathway based on the observation that Parkin and PINK1 knock-out *Drosophila melanogaster* have a similar phenotype (muscle degeneration, cell death and mitochondrial abnormalities) and that over-expression of Parkin can rescue the PINK1 knockout phenotype, but not vice versa, placing Parkin downstream of PINK1 (Clark et al., 2006; Park et al., 2006). Subsequent to these seminal contributions, PINK1 and Parkin have emerged as potential regulators for mitophagy in mammalian cells.

PINK1 is a ubiquitously expressed protein of 64 kDa that localizes to the outer membrane of mitochondria (although a proportion of the protein has been reported to possibly reside in the cytosol (Lin and Kang, 2008)). It consists of a serine/threonine kinase domain which we reported to face the cytoplasm (Zhou et al., 2008) and at least one predicted transmembrane domain, which together with the C-terminus seems to operate as a stop-transfer signal anchoring PINK1 to the mitochondrial outer membrane (MOM) (Becker et al., 2012). Under basal conditions, PINK1 appears to be rapidly turned over, keeping protein levels low (Lin and Kang, 2008). According to current models, which are primarily based on studies in cell lines such as HeLa cells and Mouse Embryonic Fibroblasts (MEFs), a portion of full-length PINK1 ($PINK1_{p64}$) is imported into the mitochondria. In the presence of mitochondrial membrane potential, it is transported first through the Translocase of the Outer Membrane (TOM) complex and seems to transiently cross the mitochondrial inner membrane via the TIM23 machinery (Becker et al., 2012). There, $PINK1_{p64}$ is believed to be cleaved by mitochondrial processing peptidase (MPP) (Greene et al., 2012), forming $PINK1_{f60}$, and then by presenilin-associated rhomboid-like protease (PARL) (Becker et al., 2012; Greene et al., 2012; Jin et al., 2010), generating a 53 kDa ($PINK1_{f53}$) fragment. Through as yet unidentified mechanisms, this $PINK1_{f53}$ fragment is retrogradely translocated to the mitochondrial surface, where it either remains attached to the mitochondrial outer membrane (MOM) or is released as a soluble protein to the cytosol and can be degraded by the proteasome (Becker et al., 2012; Jin et al., 2010). Depolarization of the mitochondrial membrane potential ($\Delta\Psi_m$), e.g. by the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) or the ionophore valinomycin, leads to a decrease in import and cleavage and a consequent rapid accumulation of full-length $PINK1_{p64}$ at the MOM (Fig. 1). The presence of full-length PINK1 on the MOM leads to recruitment of Parkin to the mitochondria (Geisler et al., 2010; Kawajiri et al., 2010; Michiorri et al., 2010; Narendra et al., 2010a,b; Vives-Bauza et al., 2010). Interestingly, both forms of PINK1 were found in high molecular weight complexes of 900 kDa (Becker et al., 2012) and 700 kDa (Becker et al., 2012; Lazarou et al., 2012) with a so far unidentified function.

Parkin is a predominantly cytosolic protein characterized by an ubiquitin-like domain at the N-terminus and a RING-between-RING domain at the C-terminus (Darios et al., 2003). It acts as an E3 ubiquitin ligase and is capable of tagging proteins for degradation at different residues including Lys48 and Lys63 (Pilsl and Winklhofer, 2012). How PINK1 is able to recruit cytosolic Parkin to the mitochondria remains unknown. Several studies have shown that PINK1 and Parkin can be co-purified, both in overexpression cell culture models and in brain tissue from either rats or humans (Kim et al., 2008; Sha et al., 2010; Shiba et al., 2009; Um et al., 2009). It is conceivable that PINK1 and Parkin interact by phosphorylating or ubiquitinating each other and although there have been reports of phosphorylation of Parkin by PINK1 and ubiquitination of PINK1 by Parkin; (Kim et al., 2008; Sha et al., 2010), we were unable to find such an interaction (Vives-Bauza et al., 2010).

After Parkin has been recruited, mitochondria are targeted for mitophagy, a specific form of macroautophagy (Wang and Klionsky, 2011). During macroautophagy, a double-membrane cup-shaped structure engulfs cytosolic content forming an autophagic vesicle (AV). This AV then fuses with lysosomes, allowing hydrolytic enzymes in what are now autophagolysosomes to degrade the contents to their biochemical components (Klionsky, 2010). Autophagy is activated under conditions of both extracellular stress such as nutrient starvation, hypoxia, overcrowding and high temperatures, and intracellular stress. Starvation induced autophagy is believed to allow cellular survival by regenerating amino acids, while basal autophagy is thought to be involved in clean-up of e.g. misfolded proteins (Cuervo, 2004).

The mechanisms by which PINK1 and Parkin regulate mitophagy have been a main focus of research in PD in recent years. Based on currently available data, we conceive of three potential scenarios for this regulation that are not necessarily mutually exclusive; 1) PINK1 and Parkin may regulate mitochondrial size by affecting fusion and fission, allowing fragmented mitochondria to be taken up in autophagic vesicles. 2) PINK1 and Parkin may skew the movement of mitochondria towards the autophagosome- and lysosome-rich perinuclear area, where the probability of being taken up by AVs is higher. 3) PINK1 and Parkin may directly recruit the autophagic machinery to damaged mitochondria for degradation. It must be noted that the majority of studies have been performed in non-neuronal cells types such as HeLa and MEF cells and that protonophores such as CCCP are frequently used to collapse membrane potential, which is thought to be a functional correlate of mitochondrial damage.

PINK1 and Parkin shift the balance towards fission

As mentioned in the Introduction, mitochondria form dynamic networks that constantly undergo fusion and fission. The core fission machinery in mammalian cells includes the cytosolic dynamin-related protein 1 (DRP1) and mitochondrial fission protein (FIS1), which is found on the MOM. Fusion is regulated primarily by the GTPase proteins mitofusins (MFNs) 1 and 2, also located on the MOM, and the dynamin-like GTPase protein optic atrophy 1 (OPA1), present in the mitochondrial intermembrane space (Liesa et al., 2009).

Fusion and fission are thought to play a role in the quality control of the mitochondrial network (Twig et al., 2008a,b). Fission, for example, may create asymmetric daughters where one mitochondrion is "healthy" and has normal membrane potential while the other has low membrane potential or contains damaged mtDNA. Twig et al. (2008a,b) found that in INS-1 cells, mitochondria that have low $\Delta\Psi_m$ after fission are six times less likely to fuse back with the network. In addition, inhibition of the fission machinery by knock-down of FIS1, use of