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INVITED REVIEW

PINK1 in the limelight: multiple functions of an eclectic protein in human health and disease

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Abstract

The gene *PINK1* [phosphatase and tensin homologue (PTEN)-induced putative kinase 1] encodes a serine/threonine kinase which was initially linked to the pathogenesis of a familial form of Parkinson's disease. Research on PINK1 has recently unravelled that its multiple functions extend well beyond neuroprotection, implicating this eclectic protein in a growing number of human pathologies, including cancer, diabetes, cardiopulmonary dysfunctions, and inflammation. Extensive studies have identified PINK1 as a crucial player in the mitochondrial quality control pathway, required to label damaged mitochondria and promote their elimination through an autophagic process (mitophagy). Mounting evidence now indicates that PINK1 activities are not restricted solely to mitophagy, and that different subcellular and even sub-mitochondrial pools of PINK1 are involved in distinct signalling cascades to regulate cell metabolism and survival. In this review, we provide a concise overview on the different functions of PINK1 and their potential role in human diseases.

Keywords: PINK1; Parkinson disease; neurodegeneration; cancer; serine-threonine kinase; mitochondria; autophagy; mitophagy

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Introduction

The *PINK1* gene was initially identified in 2001 as an up-regulated target of PTEN in endometrial cancer cells [1], but only in 2004 did this gene come into the spotlight, when recessive mutations of *PINK1* were found to cause early-onset Parkinson disease (EOPD) [2]. The large number of mutations subsequently reported in several families worldwide made *PINK1* the second commonest cause of autosomal recessive EOPD after parkin [3].

Apart from the early onset (usually less than 45 years) and the slow disease progression with sustained beneficial response to levodopa, the PINK1-related phenotype was often reported as indistinguishable from idiopathic PD [4], with occasional additional features such as dystonia at onset, levodopa-induced dyskinesias, and psychiatric disturbances [3,5]. Interestingly, *PINK1* single heterozygous mutations were detected in a number of sporadic PD patients as well as in controls displaying very mild signs of parkinsonism or subclinical manifestations, supporting the hypothesis that haploinsufficiency of *PINK1* may represent a susceptibility factor for PD [6,7].

After the first study showing a neuroprotective role against proteasomal inhibition [2], research on PINK1 has increased impressively, unravelling multiple distinct functions beyond neuroprotection, which have implicated this eclectic protein widely in cancer development, regulation of metabolism, cardiac function, and inflammation.

PINK1 processing and stability

PINK1 encodes a ubiquitously expressed 581-amino acid protein containing an N-terminal mitochondrial leader peptide (MTS), a transmembrane domain, and a vast highly conserved serine-threonine kinase domain [2,8,9]. In healthy cells, the full-length 63 kDa PINK1 protein (PINK1-FL) is imported to the inner mitochondrial membrane (IMM) via the TIM/TOM translocase complex [10]; here, PINK1-FL undergoes sequential processing operated by IMM proteases (MPP, m-AAA, ClpX, and PARL), producing an N-terminal cleaved isoform that is released into the cytosol. Cytosolic

PINK1 is then stabilized, likely through the interaction with molecular chaperones, or degraded in a proteasome-dependent manner through the N-end rule pathway [11–14]. Due to this process, endogenous PINK1 is constitutively maintained at very low levels in cells with physiologically polarized mitochondria.

Conversely, a massive, selective accumulation of PINK1-FL at the outer mitochondrial membrane (OMM) is observed following mitochondrial depolarization, misfolded protein accumulation in the mitochondrial matrix, treatment with OXPHOS inhibitors or PD-related toxins [15]. The exact mechanism by which PINK1 is stabilized on damaged mitochondria is controversial: one possibility is represented by the inactivation of IMM proteases due to the dissipation of membrane potential [16–19]; an alternative model suggests that PINK1-FL is entrapped into a 700 kDa TOM complex at the OMM, separated from the IMM proteases [10]. Finally, two novel interactors (SARM1 and TRAF6) have been implicated in PINK1 K63-linked ubiquitination and stabilization [20]. On the OMM, PINK1 undergoes dimerization and autophosphorylation, required for its complete activation and mitochondrial quality control functions [10,21–23].

It is now clear that depending on its subcellular localization, PINK1 is able to finely regulate both mitochondrial and cytosolic pathways implicated in cell survival and metabolism. In the next sections, we will briefly review the role of differently located pools of PINK1 in coordinating multiple cellular functions and their relevance to human pathology.

PINK1 functions in health and disease

PINK1 and neurodegeneration

Parkinson's disease (PD) is one of the commonest neurodegenerative disorders, affecting more than 1% of the population over 60 years; its motor phenotype, characterized by bradykinesia, rigidity, resting tremor, and postural instability, reflects the progressive loss of nigral dopaminergic neurons [24,25]. Diagnostic hallmarks of PD are Lewy bodies – cytoplasmic inclusions within surviving neurons containing α-synuclein, ubiquitin, and other proteins [26]. Autopsy studies have reported classical Lewy body pathology in *PINK1*-mutated patients, confirming that *PINK1* mutations induce a neurodegenerative process similar to PD [27,28].

Several studies have consistently demonstrated the protective role of PINK1 against neuronal death induced by several stress conditions, such as those induced by proteasomal inhibition [2,29,30], staurosporine [31–34], ceramide [35], oxidative stress [36–41], endoplasmic reticulum stress [42], mitochondrial inhibitors [43–46], and growth factor withdrawal [47,48]. More specifically related to PD, PINK1 overexpression was found to protect neurons exposed to the parkinsonian neurotoxin MPTP [49,50], and to ameliorate reduced mobility and lifespan in a *Drosophila* PD model overexpressing mutant α-synuclein [51]. A neuroprotective activity of PINK1 was implicated not only in the context of PD but also in other neurodegenerative and neuroinflammatory disorders, including Alzheimer's disease [52], multiple sclerosis [53], amyotrophic lateral sclerosis [54], and Huntington's disease [55]. Importantly, similar functional defects leading to neuronal death, mainly consisting in mitochondrial dysfunction and impairment of intracellular clearance systems, underlie all of these disorders. The efficient functioning of these pathways is essential, especially in neurons which face high levels of reactive oxygen species due to their strong reliance on mitochondrial OXPHOS; moreover, being long-living post-mitotic cells, neurons are unable to dilute the toxic effects of damaged proteins through the cell cycle, and therefore require perfectly working quality control mechanisms [56].

However, several *PINK1*-KO mouse models have failed to display loss of dopaminergic neurons or obvious PD phenotypes, suggesting the existence of compensatory mechanisms able to cope with PINK1 deficiency [57,58]. Even 2-year-old mice that were triple KO for *PINK1/parkin/DJ-1* (the three main genes causative of autosomal recessive EOPD) did not show abnormalities in dopamine metabolism or signs of neurodegeneration. These observations find a likely explanation in the mouse lifespan being too short to reach a threshold of damage sufficient to manifest an overt phenotype [59]. Indeed, dopaminergic neurodegeneration was observed in *PINK1*-KO mice upon α-synuclein- or MPTP-induced toxicity, indicating an increased susceptibility to both cellular and environmental stress [60,61]. In contrast to the mouse model, *PINK1*-KO rats present progressive nigral neurodegeneration and significant motor deficits even in basal conditions [62]. Similar observations were made in *PINK1*-mutant *Drosophila*, which displayed dopaminergic loss, locomotive dysfunction, and reduced lifespan [63–65].

In the following paragraphs, we will review the experimental evidence of the many neuroprotective activities of PINK1, both when it accumulates on depolarized mitochondria and when it is cleaved within mitochondria and then exported back into the cytosol. All of these pathways are schematically summarized in Figure 1 and Table 1.

The role of PINK1 in depolarized mitochondria

Mitochondrial quality control: mitochondrial dynamics and mitophagy Mitochondrial dysfunction plays a central role in most neurodegenerative disorders, leading to increased oxidative stress, reduced ATP production, and decreased cell survival. To preserve a population of healthy mitochondria and avoid cell death, neuronal mitochondria are subjected to stringent quality control processes, which impact on mitochondrial biogenesis, fusion/fission, trafficking, and removal. For instance, dysfunctional mitochondria inactivate their fusion machinery to avoid incorporation of altered metabolites into healthy organelles. In parallel, enhanced fission

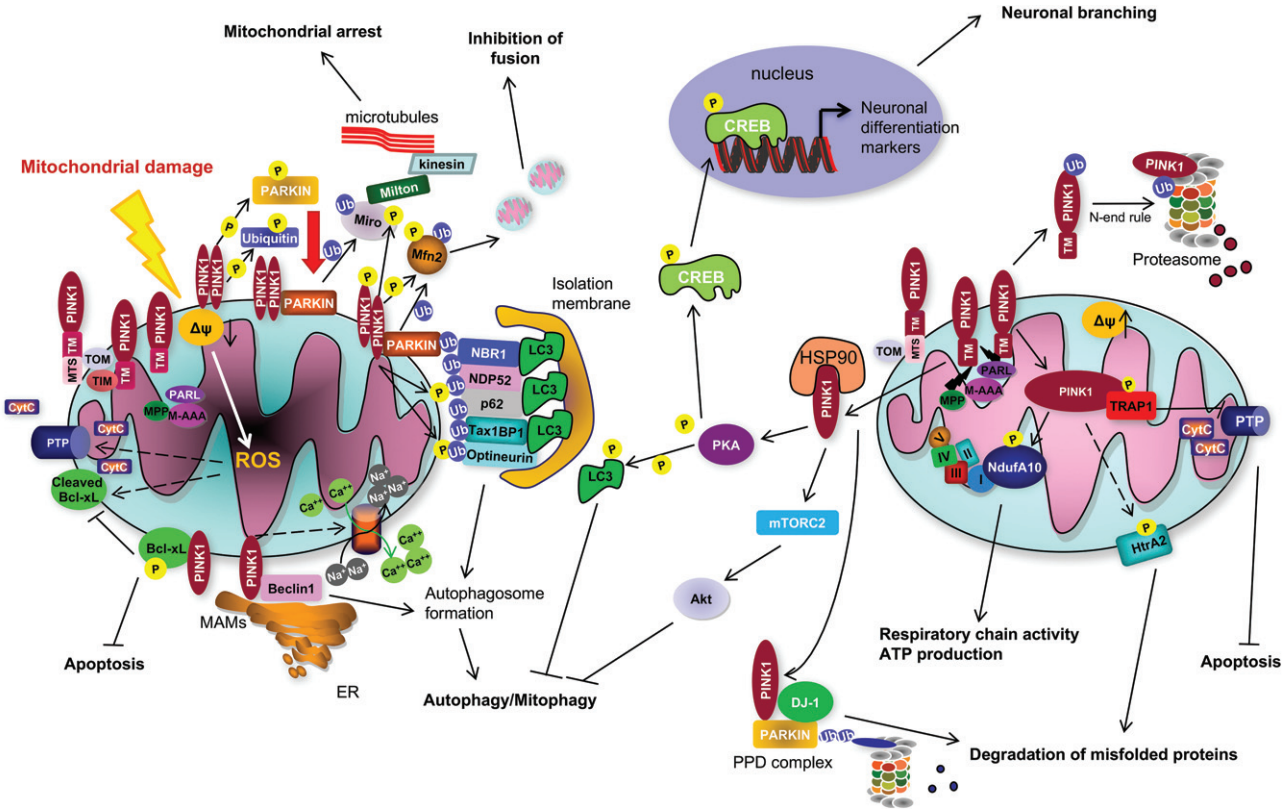

Figure 1. In healthy cells, full-length PINK1 is constitutively imported inside mitochondria and processed in a mature isoform by proteases of the inner mitochondrial membrane; at this level, PINK1 is able to sustain respiratory chain activity and ATP production by phosphorylating the complex I subunit NdufA10. Inside mitochondria, PINK1 also interacts with the mitochondrial chaperone TRAP1 and with the protease HtrA2, both involved in stress resistance mechanisms. In parallel, a pool of cleaved PINK1 is exported into the cytosol and degraded in a proteasome-dependent manner, or stabilized in functional complexes by the interaction with molecular chaperones (such as HSP90). Cytosolic PINK1 activates both the mTORC2/Akt and the PKA signalling pathways, initiating transduction cascades that culminate in autophagy/mitophagy inhibition and activation of neuronal branching. In the cytosol, PINK1 also forms a complex with parkin and DJ-1 (PPD complex), involved in the ubiquitination and proteasomal degradation of misfolded/damaged proteins. Following mitochondrial damage, proteases of the inner mitochondrial membranes are inhibited and full-length PINK1 accumulates in a dimeric form on the outer mitochondrial membrane. PINK1-mediated phosphorylation of parkin and/or ubiquitin induces parkin recruitment on the mitochondrial surface; the combined action of PINK1 and parkin, through phosphorylation, ubiquitination, and subsequent proteasomal removal of Miro and Mfn2, leads to mitochondrial arrest and inhibition of mitochondrial fusion, respectively. In this manner, dysfunctional mitochondria are put in quarantine and isolated from the healthy network before their elimination. To this aim, the extensive ubiquitination of the outer mitochondrial membrane by parkin triggers the translocation of several members of the autophagy receptors on the surface of damaged organelles, finally leading to autophagosome formation and mitophagy activation. PINK1 is also able to promote autophagosome formation, independently of parkin, by interacting with the pro-autophagic protein beclin 1 at mitochondria/ER contact sites (MAMs, mitochondria-associated membranes). At this level, PINK1 would also control the amount of intramitochondrial calcium by regulating the activity of Na^+/Ca^{2+} channels. In parallel, PINK1 protects from apoptotic cell death induced by mitochondrial depolarization by phosphorylating Bcl-xL and impairing the formation of its N-terminal cleaved pro-apoptotic fragment. Through this mechanism, PINK1 would slow down cytochrome *c* release from damaged organelles (and the consequent fatal activation of caspases), waiting for their removal through mitophagy.

generates daughter mitochondria with different fates: organelles with higher membrane potential re-enter the network, whereas depolarized mitochondria are arrested, detained in perinuclear clusters, and ultimately removed through autophagy [66,67]. Upon oxidative stress, the selective removal of oxidized mitochondrial proteins without eliminating the entire mitochondrial pool can also be mediated by a different pathway, where mitochondrial derived vesicles (MDVs) deliver the altered cargo to lysosomes for degradation [68].

PINK1 was found to act as a key biological sensor of mitochondrial dysfunction that flags depolarized mitochondria by selectively accumulating on their surface and recruiting parkin, an E3 ubiquitin-ligase also mutated in EOPD. The first evidence that PINK1 and parkin could work in a common pathway came from genetic complementation studies in *Drosophila*. Both *PINK1*- and *parkin*-KO flies displayed profound aberrations in mitochondrial morphology, characterized by enlarged/elongated or swollen organelles with fragmented cristae, and reduced mitochondrial DNA (mtDNA) content and ATP production. This phenotype could be rescued in both cases by overexpression of *parkin*, which placed it genetically downstream of *PINK1* [63–65]. Further studies revealed that the PINK1–parkin axis plays a major role in the selective degradation of depolarized mitochondria through the autophagy pathway, an essential process termed

Table 1. Functions of distinct subcellular pools of PINK1 in mitochondrial and cytosolic pathways

Functions	Pathways	Key references
PINK1-FL on the outer mitochondrial membrane		
Activation of mitophagy/autophagy	Phosphorylation of ubiquitin and recruitment of parkin	79,81,82
	Recruitment of beclin 1	92
	Recruitment of autophagy receptors	85
Regulation of mitochondrial fusion	Phosphorylation of mitofusin	83
Regulation of mitochondrial trafficking	Phosphorylation of Miro	84
Anti-apoptotic activity	Phosphorylation of Bcl-xL	46
Modulation of intramitochondrial Ca^{2+}	Regulation of Na^+/Ca^{2+} exchanger	9
	Enhancement of ER–mitochondria tethering	171
Intramitochondrial PINK1		
Regulation of complex I activity	Phosphorylation of NdufA10	111
Anti-apoptotic activity	Phosphorylation of TRAP1	36
Removal of misfolded proteins	Phosphorylation of HtrA2	114
Cytoplasmic PINK1		
Neuronal branching	PKA/CREB signalling	117
Repression of mitophagy/autophagy	PKA-dependent LC3 phosphorylation	120
	mTORC/Akt signalling	39
Removal of misfolded proteins	PPD complex formation	122

'mitophagy' [69–76]. The molecular mechanism leading to parkin recruitment on mitochondria was long debated [77–80]. Recently, three independent groups reported that ubiquitin is directly phosphorylated by PINK1 and may represent the parkin receptor on the OMM, required for its translocation [79,81,82]. The subsequent K48- and K11-linked ubiquitination of Mfn1/2 and Miro by parkin rapidly induces their proteasomal degradation, preventing mitochondrial trafficking and fusion with healthy organelles [83,84]. In parallel, K63-linked ubiquitin chains are recognized by proteins of the autophagy receptor family (p62, NDP52, NBR1, TAX1BP1, and optineurin), which bind to LC3/GABARAP family members attached to the autophagosomal membranes; in this manner, the autophagy machinery is selectively recruited in proximity of depolarized mitochondria to promote their engulfment by autophagosomes and fusion with lysosomes [85–87]. Of note, regulation of mitochondrial quality control by the PINK1–parkin axis also includes the MDV pathway [88].

Interestingly, mitophagy mediated by endogenous PINK1 and parkin was also shown to occur in mouse primary hippocampal neurons without the addition of depolarizing agents [89]. Moreover, human fibroblasts and neurons derived from induced pluripotent stem cells (iPSCs) of *PINK1*-mutated patients failed to induce parkin recruitment on mitochondria [90,91], underlying the physiological relevance of the PINK1–parkin axis in regulating mitophagy.

Parkin-independent regulation of mitophagy Besides its well-established function to recruit parkin onto damaged mitochondria, a novel role has recently emerged for PINK1 in regulating autophagy, an essential homeostatic process aimed at clearing misfolded or aggregated proteins and ensuring organelle turnover. We first showed that PINK1 promotes both basal and starvation-induced autophagy, and directly interacts with the key pro-autophagic protein beclin 1 [92]. More recently, we found that upon mitochondrial depolarization, PINK1 selectively accumulates to specific sites of contact between endoplasmic reticulum (ER) and mitochondria (MAMs, mitochondria-associated membranes), where it recruits beclin 1 to initiate autophagosome formation [171]. This is an essential step to form the membranes that will engulf damaged mitochondria or other cellular debris, before their fusion with lysosomes for enzymatic digestion [93], and is pivotal not only for mitophagy but for all autophagic processes. Moreover, a recent study on depolarized mitochondria showed that PINK1 is also able to activate a parkin-independent cascade, leading to the recruitment of several early autophagy factors to focal spots proximal to mitochondria [85]. These findings clearly suggest that PINK1 can activate mitophagy (and possibly autophagy at large) through a number of distinct and parallel pathways.

Anti-apoptotic function While part of the anti-apoptotic activity of PINK1 could be explained by the mitophagic removal of damaged mitochondria before they trigger the apoptotic cascade, other mechanisms have also been advocated. We recently demonstrated that upon mitochondrial depolarization induced by carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), PINK1 directly interacts with and phosphorylates serine 62 of Bcl-xL, a main anti-apoptotic protein of the Bcl-2 family highly expressed in neuronal cells.

The anti-apoptotic function of Bcl-xL is largely mediated by its N-terminal BH4 domain, which is able to preserve the mitochondrial membrane potential and block cytochrome *c* release by inhibiting the voltage-dependent anion channel (VDAC) activity. Pro-apoptotic stimuli such as CCCP induce cleavage of Bcl-xL at aspartate 61, generating a C-terminal fragment that potently stimulates apoptosis, and this cleavage is hampered by PINK1-mediated phosphorylation of Bcl-xL at the nearby serine 62 [46]. Accordingly, pharmacological activation of PINK1 by the ATP analogue kinetin triphosphate (KTP) was found to enhance

PINK1-mediated Bcl-xL phosphorylation and reduce CCCP-induced apoptosis [94].

Regulation of mitochondrial calcium homeostasis Alterations of intramitochondrial calcium levels have been reported in several neurodegenerative disorders, including PD [95]. Different studies demonstrated a role for PINK1 in the regulation of this pathway. Depletion of PINK1 in both human and mouse neurons increased the amount of mitochondrial calcium, likely related to impairment of the Na^+/Ca^{2+} exchanger and consequent reduction of calcium efflux from mitochondria [96]. While physiological levels of mitochondrial calcium sustain oxidative metabolism and ATP production, excessive amounts of intramitochondrial calcium are detrimental for respiratory chain function, inducing collapse of mitochondrial membrane potential, increased ROS production, and mPTP opening [97]. In line with this, treatment with ruthenium red (a well-known inhibitor of mitochondrial calcium uptake) significantly rescued the mitochondrial damage induced by *PINK1* mutations [98]. Our recent finding that upon mitochondrial depolarization, PINK1 selectively accumulates at the MAMs, which are the sites where calcium is exchanged through the uniporter channel [99], strongly supports a central role for PINK1 in regulating calcium homeostasis, for which further studies are clearly warranted.

The role of PINK1 within and outside mitochondria

Most of the preceding data relate to experimental conditions of massive and acute mitochondrial depolarization, which are not expected to occur in more physiological settings such as ageing neurons. Yet even cleaved PINK1 has been found to play neuroprotective roles both within the mitochondria and after cytosolic export.

Modulation of the respiratory chain activity Complex I activity is impaired by parkinsonian stressors such as MPTP and rotenone, and was found to be reduced in the brains of sporadic PD patients [100,101]. Many studies implicated PINK1 in mitochondrial metabolism, as demonstrated by impaired electron transport chain activity, reduced oxygen consumption, and increased ROS in PINK1-depleted cells [37,44,58,102–104]. In particular, decreased complex I activity was observed in several *PINK1*-null models, explaining the higher sensitivity of PINK1-deficient cells to complex I inhibitors [37,60,105–110]. One mechanism through which PINK1 could regulate complex I involves its direct phosphorylation of NADH dehydrogenase ubiquinone 1 alpha subcomplex 10 (NdufA10), a subunit required for ubiquinone reductase activity [111]. Another explanation may reside in the ability of PINK1 to regulate mitochondrial targeting and translation of certain nuclear mRNAs encoding respiratory chain components, including the complex I subunits C-I 30 and ND75 [112].

Other intramitochondrial targets of PINK1 In addition to substrates located on the OMM, PINK1 was found to interact with intramitochondrial proteins, such as the chaperone TRAP1 and the serine protease HtrA2. PINK1-mediated phosphorylation of TRAP1 protects mitochondria against oxidative stress-induced apoptosis [36]. According to this notion, TRAP1 overexpression in *PINK1*-mutant *Drosophila* neurons rescued mitochondrial dysfunction and cell death [113]. Increased protection against mitochondrial stress could also be mediated by phosphorylation of HtrA2 and activation of its proteolytic activity, which in turn would remove damaged mitochondrial proteins. Indeed, mice lacking HtrA2 displayed accumulation of misfolded proteins and increased ROS levels in brain mitochondria, and loss of function *HtrA2* mutations lead to neuronal death in humans as well. Finally, *HtrA2* represents a susceptibility factor for PD, and reduced levels of phospho-HtrA2 were observed in the brains of *PINK1*-mutated PD patients [114,115].

Neuroprotective functions of cytosolic PINK1 Growing evidence indicates that N-terminal truncated cytosolic PINK1 could promote neuron survival and differentiation [116]. Haque *et al* first demonstrated that a PINK1 construct lacking the mitochondrial targeting sequence (ΔN-PINK1), mainly localized in the cytosol, protected neurons against MPTP [49]. In line with this, cytosolic PINK1 rescued the reduced dendritic branching observed in PINK1-deficient neurons, likely by enhancing anterograde transport and the density of mitochondria in dendrites. Transfection of ΔN-PINK1 (but not of OMM-sequestered PINK1) in undifferentiated SH-SY5Y cells induced the expression of neuronal differentiation markers and promoted neurite extension, indicating divergent roles for cytosolic and mitochondrial PINK1 in modulating this phenotype [117]. Protein kinase A (PKA), which is known to regulate neurite outgrowth, spine formation, and synaptogenesis through activation of the CREB transcription factor, seems to be involved in this process [118], as pharmacological inhibition of PKA represses dendritic branching induced by overexpression of cytosolic PINK1 [117].

PINK1 also activates the cytosolic mTORC2/Akt pathway, which is known to play a fundamental role in neuron survival by sustaining multiple trophic effects on dopaminergic cells, including axonal growth [39,119].

In addition, cytosolic PINK1 was reported to suppress macroautophagy and mitophagy induced by chronic deficiency of endogenous PINK1 [120,121]; LC3 phosphorylation, Akt activation or parkin sequestration in the cytosol could be the downstream effectors.

In the cytosol, PINK1 was also reported to form an E3-ligase complex with both parkin and DJ-1 (PPD complex), promoting ubiquitination and proteasomal degradation of aberrant parkin substrates such as synphilin-1, and possibly of parkin itself [122].

Interestingly, both mitochondrial and cytosolic PINK1 retain the ability to protect against apoptosis and block neuronal death induced by several stress conditions; this supports the concept that different subcellular pools of PINK1 regulate distinct biological functions, with the common goal of promoting neuronal function and survival.

PINK1 and cancer

As mentioned before, *PINK1* was first discovered as a gene modulated by the tumour suppressor PTEN in a cancer cell model [1]. Later on, strong expression of PINK1 was observed in melanoma and colon carcinoma mouse cancer cell lines displaying high metastatic potential [123]. The subsequent investigations on PINK1 functions further reinforced these pioneering findings, highlighting its involvement in several aspects of cancer biology and metabolism. Interestingly, many of the pathways involved in neurodegeneration were also implicated in oncogenic transformation and cancer progression. According to this notion, epidemiological studies revealed a reduced risk of certain cancers in patients with PD [124]. Moreover, PINK1 has been identified as a fundamental element for tumour cell survival and chemoresistance in two independent RNA interference screens, making it a potential therapeutic target for cancer treatment [125–127], and a recent meta-analysis in human ovarian carcinoma revealed that high *PINK1* mRNA expression correlates with a poor outcome [128]. These findings are supported by several studies showing a major role for PINK1 in sustaining cell proliferation and resistance to death, by impacting fundamental processes such as cell cycle control, apoptosis, regulation of protein degradation systems, mitochondrial homeostasis, and cell metabolism.

Cell cycle regulation

Several studies have reported a role for PINK1 in processes associated with cell cycle regulation. O'Flanagan *et al* demonstrated that PINK1 is a positive regulator of cell cycle progression, promoting cell division and growth by altering G2/M and G0/G1 checkpoints. Deletion of PINK1 in both mouse embryonic fibroblasts and cancer cells induced cell cycle arrest in the G2/M phase at cytokinesis and accumulation of multinucleated cells, indicative of defective mitosis; in turn, this translated into reduced cell proliferation, colony formation, and migration. Cell cycle alterations induced by PINK1 silencing were linked to excessive mitochondrial fragmentation, confirming the relevance of mitochondrial dynamics for cell cycle progression [129]. The expression, mitochondrial recruitment, and activity of Drp1, a key mediator of mitochondrial fission, are finely regulated throughout mitosis, with a strong increase during metaphase, to allow the equal distribution of mitochondria to daughter cells [130]. In PINK1-deficient cells, Drp1 levels were significantly higher compared with control cells, explaining the increased mitochondrial fragmentation and the cytokinesis defect, and Drp1 knockdown significantly rescued multinucleation caused by PINK1 deficiency [129]. The role of PINK1 in cell cycle progression could explain why PINK1 silencing was able to sensitize cancer cells to chemotherapeutic drugs targeting mitotic cells [125]. In this light, inhibition of PINK1 combined with paclitaxel treatment might be effective synergistically to kill cancer cells.

Protection from apoptosis

Extensive literature has consistently pointed towards a protective role of PINK1 against apoptosis induced by different stressors, including chemotherapy-related compounds (see above). The increased cell death observed in PINK1-depleted cells is intimately linked to mitochondrial dysfunction, and therefore to the intrinsic apoptotic pathway. Indeed, by affecting many aspects of mitochondrial homeostasis, such as mtDNA levels, mitochondrial membrane potential, electron transport chain, and mitochondrial calcium, the down-regulation of PINK1 results in decreased ATP synthesis and increased oxidative stress. In turn, ROS production facilitates the opening of mitochondrial permeability transition pore (mPTP), inducing cytochrome *c* release and activation of the apoptotic cascade [29,33,44,96,102]. A direct effect of PINK1 on proteins belonging to the family of the apoptosis regulator Bcl-2 has also been reported. For instance, PINK1 overexpression inhibits the activation of the pro-apoptotic protein Bax upon proteasomal stress [30], whereas its silencing up-regulates Bax and prevents the activation of Bcl-xL [127]. Moreover, the direct binding and phosphorylation of Bcl-xL (as discussed above) [46] could represent an anti-apoptotic mechanism involved not only in neurodegeneration but also in cancer. All of these findings suggest that compounds targeting mitochondrial function coupled to PINK1 depletion could increase apoptosis, and therefore represent a potential target for cancer treatment.

Regulation of protein degradation systems

Alterations in the pathways of intracellular clearance are directly related to the pathogenesis of cancer. In recent years, the ubiquitin proteasome system (UPS) has been recognized as an essential mechanism that cancer cells employ to alter the balance between cell growth and death and to induce drug resistance. In fact, many UPS targets are regulatory proteins involved in cell cycle progression, DNA damage response, signal transduction, and cell metabolism [131].

The role of autophagy in cancer is more complex and debated. On the one hand, autophagy may act as a tumour suppressor mechanism, by preventing the accumulation of misfolded proteins and damaged organelles, and limiting DNA damage and chromosomal instability. On the other hand, the ability of autophagy to sustain cell survival in hypoxic conditions or under nutrient deprivation definitely supports tumour survival [132].

Several inhibitors of both UPS and autophagy have been approved for cancer treatment, confirming that repression of these protein degradation pathways represents a promising anti-cancer strategy. Thus, PINK1 could play a key role in regulating these cellular clearance systems, not only in the context of neurodegeneration but also in the context of cancer. This has already been shown in cancer cell models, in which PINK1 deficiency impaired both proteasome activity

and stress-induced autophagy, resulting in reduced cancer cell survival [48,122].

However, cytosolic PINK1 was found to suppress autophagy and mitophagy (see above). This apparent discrepancy is paralleled by the controversial role of mitophagy in cancer. For tumours relying on mitochondrial activity to sustain their proliferation, inhibition of mitophagy represents a valid approach to induce mitochondrial dysfunction and promote cancer cell death: drugs affecting mitochondrial function would increase the need for functional mitophagy; therefore, the combined action of mitophagy inhibitors may provide additional benefits in terms of tumour suppression. However, mitophagy inhibition induced by parkin deficiency was associated with the development of hepatocellular carcinoma and lymphomas, indicating that some cancers are still able to survive despite the absence of functional mitochondria [133].

Effect on mitochondrial and cell metabolism

Cancer cells usually rely on both glycolysis and mitochondrial metabolism to sustain their proliferation. Mitochondria provide cells not only with ATP but also with all the TCA cycle intermediates necessary to synthesize lipids, amino acids, and nucleotides, which are all essentials to satisfy the bioenergetics demand of cancer. As a consequence, cancer cells display a strong activation of the electron transport chain [134].

The role of PINK1 in regulating complex I activity has already been mentioned before. Moreover, at least in cancer cells, PINK1 could facilitate the mitochondrial uptake of Ca^{2+}, which in turn allosterically triggers several TCA cycle enzymes to sustain oxidative metabolism; accordingly, depletion of PINK1 significantly reduces Ca^{2+} entry into the mitochondrial matrix, decreasing mitochondrial metabolism [135]. Of note, a metabolic shift leading to enhanced ATP production by glycolysis, followed by increased lactic acidosis, was observed in PINK1-depleted or -mutant cells [136–138]. Mechanistically, PINK1 deficiency could induce ROS-mediated activation of HIF1α, leading to increased glucose metabolism [139]. Loss of PINK1 also induced compensatory metabolic reprogramming to fuel nucleotide pools and promote mitochondrial biogenesis [140].

Tumour suppressor functions

Although the main functions of PINK1 clearly point to a proto-oncogenic role, tumour suppressive properties have also been proposed in some contexts. The *PINK1* gene is located on chromosome 1p36, a region frequently deleted in several cancers [141]; *PINK1* expression is induced by PTEN and FOXO3a [1,47], two tumour suppressors, and parkin, which acts downstream of PINK1 itself, is also an oncosuppressor [142]. Meta-analysis of *PINK1* mRNA levels in cancer datasets revealed a significant decrease of *PINK1* expression in liver and renal carcinoma, as well as in several primary brain tumours; moreover, *PINK1* heterozygous mutations have been identified in endometrial, oesophageal, and ovarian cancer, as well as in neuroblastoma [38,143,144]. The discrepancy between oncogenic and oncosuppressor activities of PINK1 could be explained by the metabolic reprogramming of cancer cells lacking PINK1 [138]. In parallel, PINK1 depletion significantly increased mitochondrial ROS production, known to activate signalling pathways close to the mitochondria to promote cancer cell proliferation and tumourigenesis [145]. Accordingly, treatment of PINK1 knockdown cells with antioxidant compounds suppressed ROS and blocked the glycolytic shift and cancer cell growth [143].

PINK1 and dysmetabolism

Mitochondrial dysfunction also represents a main feature of obesity and type 2 diabetes: altered mitochondrial dynamics, reduced mitochondrial biogenesis, decreased OXPHOS, and excessive ROS production have all been proposed to play a key role in insulin resistance [146].

Scheele *et al* first observed reduced PINK1 expression levels in the skeletal muscle of type 2 diabetic patients [147]. This was also seen in the hearts of type 1 diabetics [148] and in diabetic brains, the latter leading to lipid droplet accumulation and impaired oxidation of mitochondrial fatty acids [149]. Further confirming a role for PINK1 in lipid metabolism, PINK1-deficient primary adipocytes display decreased expression of the fatty acid binding protein 4 (Fabp4), a lipid chaperone associated with reduced lipolysis, insulin resistance, and obesity [150].

At a metabolic level, loss of PINK1 significantly inhibits glucose uptake by pancreatic β-cells and primary intact islets, and is accompanied by increased insulin secretion and enhanced glucose tolerance [151]. Importantly, the elevated glucose levels resulting from PINK1 deficiency suppress autophagy and induce β-cell apoptosis, an effect that can be rescued by PINK1 overexpression [152]. Supporting a protective role for PINK1 for β-cells under hyperglycaemic conditions, increased expression of both PINK1 and parkin was observed in the adipose tissue of mice subjected to a prolonged high-fat diet; this was followed by activation of mitophagy, aimed at avoiding oxidative stress and the development of diabetes [153]. In this light, pharmacological interventions targeting mitochondrial quality control may represent promising approaches for the treatment of diabetes and obesity.

PINK1 and heart defects

The heart is highly dependent on mitochondria to produce ATP necessary for its function, and preservation of a functional mitochondrial pool is fundamental to keep cardiomyocytes healthy [154]. Billia *et al* first demonstrated an essential role for PINK1 in heart muscle homeostasis: *PINK1* KO mice developed left ventricular dysfunction and cardiac hypertrophy, leading to pressure overload-induced heart

failure. Mitochondria from *PINK1* KO hearts displayed altered morphology, reduced mitochondrial membrane potential, and decreased OXPHOS, which in turn induced oxidative stress and increased cardiomyocyte apoptosis [155,156]. Cardiac hypertrophy and contractile anomalies were also observed in the hearts from mice genetically ablated for either *PTEN* or *FoxO1/3*, the two main transcriptional regulators of *PINK1* expression; this was accompanied by reduced autophagy, decreased antioxidant capacity, and induction of apoptosis [157,158]. Mitochondrial fragmentation, apoptosis, and myocardial infarction were also observed following E2F1-mediated activation of miR-421, known to suppress *PINK1* translation in cardiomyocytes [159]. Through ROS production and mtDNA release in the cytosol, damaged mitochondria from cardiomyocytes subjected to myocardial infarction or acute ischaemia/reperfusion injury may also stimulate cytokine production and activate the inflammatory response; in this light, removal of dysfunctional mitochondria by the PINK1–parkin axis could represent a key cardioprotective mechanism before they cause further damage. Consistent with this hypothesis, mouse hearts lacking functional mitophagy developed cardiac defects with age [80]. Finally, Gong *et al* recently discovered that PINK1–parkin-dependent mitophagy is responsible for the degradation of mitochondria in embryonic cardiomyocytes, regulating the metabolic transition from fetal heart to adult heart [160].

PINK1 and inflammation

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease characterized by declining lung function and inflammation, mainly in response to cigarette smoke (CS), that triggers ROS generation in the airway epithelium. Therefore, the production of antioxidant scavengers, the remodelling of the mitochondrial network, and mitophagy represent key mechanisms to counteract oxidative stress and avoid apoptosis. According to this notion, PINK1 expression was found to be higher in COPD patients than in control smokers [161–163].

The role of PINK1 and CS-induced mitophagy in COPD is controversial: findings from Ito *et al* suggested a protective role for PINK1/parkin-mediated mitophagy against COPD development, with PINK1 loss increasing ROS production and senescence in primary human bronchial epithelial cells [164]. Impaired mitophagy and cellular senescence in the lungs of smokers and COPD patients were also reported by a different group [163]. In cultured pulmonary epithelial cells and murine models, however, PINK1-induced mitophagy seemed to be detrimental for lung cells exposed to CS, leading to activation of the necroptosis pathway; in fact, genetic depletion of PINK1 or administration of necroptosis inhibitors protected against mitochondrial dysfunction, airspace enlargement, mucociliary clearance disruption, and CS-induced cell death [162].

A role for PINK1 was also proposed in the development of lung fibrosis [165]. Knockdown of PINK1 in lung epithelial cells resulted in defective mitophagy, leading to the accumulation of dysfunctional mitochondria and the expression of pro-fibrotic factors; accordingly, low expression of PINK1 was observed in alveolar type II cells from idiopathic pulmonary fibrosis patients and in ageing lungs [166,167].

Finally, PINK1 has also been implicated in the pathogenesis of liver inflammation due to infection with hepatitis B (HBV) or C viruses (HCV), often evolving into chronic liver disease, steatosis, cirrhosis, and hepatocellular carcinoma [168]. Alteration of mitochondrial quality control and metabolism is frequently associated with HV infection, and mitophagy represents a crucial mechanism to promote cell survival and virus persistence. Both HBV and HCV were found to induce PINK1 expression and mitochondrial translocation of parkin, while PINK1 or parkin silencing impaired HV-induced mitophagy and delayed virus replication [169,170].

Conclusions and future perspectives

Largely studied in the context of PD, PINK1 has progressively been identified as a key protein, not only for neuronal survival but also for the homeostasis of several other tissues. It is becoming increasingly clear that the regulation of mitochondrial quality control, representing the most explored and attractive face of PINK1-mediated effects, operates together with other pro-survival pathways controlled by different subcellular pools of PINK1; this consideration suggests that several actors and pathways are simultaneously affected by loss-of-function mutations in the *PINK1* gene, and these should be taken into account when designing therapeutic approaches aimed at restoring PINK1 activity. In this light, research on PINK1 needs to extend well beyond mitophagy and neurodegeneration to explore other aspects of PINK1 signalling, including cell metabolism and regulation of nuclear gene expression. With the help of high-throughput 'omics' approaches, this line of research is expected to allow the identification of specific metabolic signatures, transcriptional programmes, and protein interaction networks operating in physiological conditions and altered in the context of human diseases caused by alterations of PINK1 expression. With these notions, the modulation of PINK1 function in different cellular contexts may represent a promising therapeutic strategy, not only for PD but also for cancer and other human disorders.

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Author contribution statement

Both authors contributed by searching the literature and drafting the text and figure.

References

1. Unoki M, Nakamura Y. Growth-suppressive effects of *BPOZ* and *EGR2*, two genes involved in the *PTEN* signaling pathway. *Oncogene* 2001; **20:** 4457–4465.
2. Valente EM, Abou-Sleiman PM, Caputo V, *et al.* Hereditary early-onset Parkinson's disease caused by mutations in *PINK1*. *Science* 2004; **304:** 1158–1160.
3. Bonifati V. Genetics of Parkinson's disease – state of the art, 2013. *Parkinsonism Relat Disord* 2014; **20**(suppl 1): S23–S28.
4. Albanese A, Valente EM, Romito LM, *et al.* The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. *Neurology* 2005; **64:** 1958–1960.
5. Ricciardi L, Petrucci S, Guidubaldi A, *et al.* Phenotypic variability of PINK1 expression: 12 years' clinical follow-up of two Italian families. *Mov Disord* 2014; **29:** 1561–1566.
6. Marongiu R, Ferraris A, Ialongo T, *et al.* PINK1 heterozygous rare variants: prevalence, significance and phenotypic spectrum. *Hum Mutat* 2008; **29:** 565.
7. Corti O, Lesage S, Brice A. What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiol Rev* 2011; **91:** 1161–1218.
8. Silvestri L, Caputo V, Bellacchio E, *et al.* Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum Mol Genet* 2005; **14:** 3477–3492.
9. Zhou C, Huang Y, Shao Y, *et al.* The kinase domain of mitochondrial PINK1 faces the cytoplasm. *Proc Natl Acad Sci U S A* 2008; **105:** 12022–12027.
10. Lazarou M, Jin SM, Kane LA, *et al.* Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev Cell* 2012; **22:** 320–333.
11. Lin W, Kang UJ. Characterization of PINK1 processing, stability, and subcellular localization. *J Neurochem* 2008; **106:** 464–474.
12. Moriwaki Y, Kim YJ, Ido Y, *et al.* L347P PINK1 mutant that fails to bind to Hsp90/Cdc37 chaperones is rapidly degraded in a proteasome-dependent manner. *Neurosci Res* 2008; **61:** 43–48.
13. Weihofen A, Ostaszewski B, Minami Y, *et al.* Pink1 Parkinson mutations, the Cdc37/Hsp90 chaperones and Parkin all influence the maturation or subcellular distribution of Pink1. *Hum Mol Genet* 2008; **17:** 602–616.
14. Yamano K, Youle RJ. PINK1 is degraded through the N-end rule pathway. *Autophagy* 2013; **9:** 1758–1769.
15. Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. *Autophagy* 2013; **9:** 1750–1757.
16. Jin SM, Lazarou M, Wang C, *et al.* Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol* 2010; **191:** 933–942.
17. Deas E, Plun-Favreau H, Gandhi S, *et al.* PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet* 2011; **20:** 867–879.
18. Meissner C, Lorenz H, Weihofen A, *et al.* The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem* 2011; **117:** 856–867.
19. Greene AW, Grenier K, Aguileta MA, *et al.* Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep* 2012; **13:** 378–385.
20. Murata H, Sakaguchi M, Kataoka K, *et al.* SARM1 and TRAF6 bind to and stabilize PINK1 on depolarized mitochondria. *Mol Biol Cell* 2013; **24:** 2772–2784.
21. Okatsu K, Oka T, Iguchi M, *et al.* PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nature Commun* 2012; **3:** 1016.
22. Okatsu K, Uno M, Koyano F, *et al.* A dimeric PINK1-containing complex on depolarized mitochondria stimulates Parkin recruitment. *J Biol Chem* 2013; **288:** 36372–36384.
23. Aerts L, Craessaerts K, De Strooper B, *et al.* PINK1 kinase catalytic activity is regulated by phosphorylation on serines 228 and 402. *J Biol Chem* 2015; **290:** 2798–2811.
24. Lang AE, Lozano AM. Parkinson's disease. *First of two parts. N Engl J Med* 1998; **339:** 1044–1053.
25. Lang AE, Lozano AM. Parkinson's disease. *Second of two parts. N Engl J Med* 1998; **339:** 1130–1143.
26. Spillantini MG, Crowther RA, Jakes R, *et al.* α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci U S A* 1998; **95:** 6469–6473.
27. Gandhi S, Muqit MM, Stanyer L, *et al.* PINK1 protein in normal human brain and Parkinson's disease. *Brain* 2006; **129:** 1720–1731.
28. Samaranch L, Lorenzo-Betancor O, Arbelo JM, *et al.* PINK1-linked parkinsonism is associated with Lewy body pathology. *Brain* 2010; **133:** 1128–1142.
29. Wang HL, Chou AH, Yeh TH, *et al.* PINK1 mutants associated with recessive Parkinson's disease are defective in inhibiting mitochondrial release of cytochrome *c*. *Neurobiol Dis* 2007; **28:** 216–226.
30. Klinkenberg M, Thurow N, Gispert S, *et al.* Enhanced vulnerability of PARK6 patient skin fibroblasts to apoptosis induced by proteasomal stress. *Neuroscience* 2010; **166:** 422–434.
31. Petit A, Kawarai T, Paitel E, *et al.* Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* 2005; **280:** 34025–34032.
32. Gelmetti V, Ferraris A, Brusa L, *et al.* Late onset sporadic Parkinson's disease caused by *PINK1* mutations: clinical and functional study. *Mov Disord* 2008; **23:** 881–885.
33. Wood-Kaczmar A, Gandhi S, Yao Z, *et al.* PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS One* 2008; **3:** e2455.
34. Akundi RS, Zhi L, Bueler H. PINK1 enhances insulin-like growth factor-1-dependent Akt signaling and protection against apoptosis. *Neurobiol Dis* 2012; **45:** 469–478.
35. Rojas-Charry L, Cookson MR, Nino A, *et al.* Downregulation of Pink1 influences mitochondrial fusion–fission machinery and sensitizes to neurotoxins in dopaminergic cells. *Neurotoxicology* 2014; **44:** 140–148.
36. Pridgeon JW, Olzmann JA, Chin LS, *et al.* PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol* 2007; **5:** e172.
37. Morais VA, Verstreken P, Roethig A, *et al.* Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. *EMBO Mol Med* 2009; **1:** 99–111.
38. Berthier A, Navarro S, Jimenez-Sainz J, *et al.* PINK1 displays tissue-specific subcellular location and regulates apoptosis and cell growth in breast cancer cells. *Hum Pathol* 2011; **42:** 75–87.
39. Murata H, Sakaguchi M, Jin Y, *et al.* A new cytosolic pathway from a Parkinson disease-associated kinase, BRPK/PINK1: activation of AKT via mTORC2. *J Biol Chem* 2011; **286:** 7182–7189.
40. Chien WL, Lee TR, Hung SY, *et al.* Impairment of oxidative stress-induced heme oxygenase-1 expression by the defect of

Parkinson-related gene of PINK1. *J Neurochem* 2011; **117:** 643–653.
41. Choi HK, Choi Y, Kang H, *et al.* PINK1 positively regulates HDAC3 to suppress dopaminergic neuronal cell death. *Hum Mol Genet* 2015; **24:** 1127–1141.
42. Li L, Hu GK. Pink1 protects cortical neurons from thapsigargin-induced oxidative stress and neuronal apoptosis. *Biosci Rep* 2015; **35:** e00174.
43. Deng H, Jankovic J, Guo Y, *et al.* Small interfering RNA targeting the PINK1 induces apoptosis in dopaminergic cells SH-SY5Y. *Biochem Biophys Res Commun* 2005; **337:** 1133–1138.
44. Gegg ME, Cooper JM, Schapira AH, *et al.* Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *PLoS One* 2009; **4:** e4756.
45. Wang HL, Chou AH, Wu AS, *et al.* PARK6 PINK1 mutants are defective in maintaining mitochondrial membrane potential and inhibiting ROS formation of substantia nigra dopaminergic neurons. *Biochim Biophys Acta* **2011**; 1812**:** 674–684.
46. Arena G, Gelmetti V, Torosantucci L, *et al.* PINK1 protects against cell death induced by mitochondrial depolarization, by phosphorylating Bcl-xL and impairing its pro-apoptotic cleavage. *Cell Death Differ* 2013; **20:** 920–930.
47. Mei Y, Zhang Y, Yamamoto K, *et al.* FOXO3a-dependent regulation of Pink1 (Park6) mediates survival signaling in response to cytokine deprivation. *Proc Natl Acad Sci U S A* 2009; **106:** 5153–5158.
48. Parganlija D, Klinkenberg M, Dominguez-Bautista J, *et al.* Loss of PINK1 impairs stress-induced autophagy and cell survival. *PLoS One* 2014; **9:** e95288.
49. Haque ME, Thomas KJ, D'Souza C, *et al.* Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. *Proc Natl Acad Sci U S A* 2008; **105:** 1716–1721.
50. Sallinen V, Kolehmainen J, Priyadarshini M, *et al.* Dopaminergic cell damage and vulnerability to MPTP in Pink1 knockdown zebrafish. *Neurobiol Dis* 2010; **40:** 93–101.
51. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a *Drosophila* model of Parkinson's disease. *Genome* 2008; **51:** 1040–1046.
52. Shen J. Impaired neurotransmitter release in Alzheimer's and Parkinson's diseases. *Neurodegener Dis* 2010; **7:** 80–83.
53. Wilhelmus MM, van der Pol SM, Jansen Q, *et al.* Association of Parkinson disease-related protein PINK1 with Alzheimer disease and multiple sclerosis brain lesions. *Free Radic Biol Med* 2011; **50:** 469–476.
54. Knippenberg S, Sipos J, Thau-Habermann N, *et al.* Altered expression of DJ-1 and PINK1 in sporadic ALS and in the SOD1(G93A) ALS mouse model. *J Neuropathol Exp Neurol* 2013; **72:** 1052–1061.
55. Khalil B, El Fissi N, Aouane A, *et al.* PINK1-induced mitophagy promotes neuroprotection in Huntington's disease. *Cell Death Dis* 2015; **6:** e1617.
56. Tooze SA, Schiavo G. Liaisons dangereuses: autophagy, neuronal survival and neurodegeneration. *Curr Opin Neurobiol* 2008; **18:** 504–515.
57. Kitada T, Pisani A, Porter DR, *et al.* Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. *Proc Natl Acad Sci U S A* 2007; **104:** 11441–11446.
58. Gispert S, Ricciardi F, Kurz A, *et al.* Parkinson phenotype in aged PINK1-deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. *PLoS One* 2009; **4:** e5777.
59. Kitada T, Tong Y, Gautier CA, *et al.* Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. *J Neurochem* 2009; **111:** 696–702.
60. Haque ME, Mount MP, Safarpour F, *et al.* Inactivation of *Pink1* gene *in vivo* sensitizes dopamine-producing neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and can be rescued by autosomal recessive Parkinson disease genes, *Parkin* or *DJ-1*. *J Biol Chem* 2012; **287:** 23162–23170.
61. Oliveras-Salva M, Macchi F, Coessens V, *et al.* Alpha-synuclein-induced neurodegeneration is exacerbated in PINK1 knockout mice. *Neurobiol Aging* 2014; **35:** 2625–2636.
62. Dave KD, De Silva S, Sheth NP, *et al.* Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiol Dis* 2014; **70:** 190–203.
63. Clark IE, Dodson MW, Jiang C, *et al. Drosophila pink1* is required for mitochondrial function and interacts genetically with *parkin*. *Nature* 2006; **441:** 1162–1166.
64. Park J, Lee SB, Lee S, *et al.* Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 2006; **441:** 1157–1161.
65. Yang Y, Gehrke S, Imai Y, *et al.* Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc Natl Acad Sci U S A* 2006; **103:** 10793–10798.
66. Twig G, Hyde B, Shirihai OS. Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. *Biochim Biophys Acta* **2008**; 1777**:** 1092–1097.
67. Ni HM, Williams JA, Ding WX. Mitochondrial dynamics and mitochondrial quality control. *Redox Biol* 2015; **4:** 6–13.
68. Soubannier V, McLelland GL, Zunino R, *et al.* A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol* 2012; **22:** 135–141.
69. Geisler S, Holmstrom KM, Skujat D, *et al.* PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nature Cell Biol* 2010; **12:** 119–131.
70. Narendra DP, Jin SM, Tanaka A, *et al.* PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 2010; **8:** e1000298.
71. Vives-Bauza C, Zhou C, Huang Y, *et al.* PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci U S A* 2010; **107:** 378–383.
72. Kawajiri S, Saiki S, Sato S, *et al.* PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy. *FEBS Lett* 2010; **584:** 1073–1079.
73. Matsuda N, Sato S, Shiba K, *et al.* PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* 2010; **189:** 211–221.
74. Abeliovich A. Parkinson's disease: pro-survival effects of PINK1. *Nature* 2007; **448:** 759–760.
75. Vincow ES, Merrihew G, Thomas RE, *et al.* The PINK1–Parkin pathway promotes both mitophagy and selective respiratory chain turnover *in vivo*. *Proc Natl Acad Sci U S A* 2013; **110:** 6400–6405.
76. Ziviani E, Tao RN, Whitworth AJ. *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 2010; **107:** 5018–5023.
77. Kondapalli C, Kazlauskaite A, Zhang N, *et al.* PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol* 2012; **2:** 120080.
78. Shiba-Fukushima K, Imai Y, Yoshida S, *et al.* PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Sci Rep* 2012; **2:** 1002.
79. Kane LA, Lazarou M, Fogel AI, *et al.* PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol* 2014; **205:** 143–153.

80. Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* 2013; **340:** 471–475.
81. Kazlauskaite A, Kondapalli C, Gourlay R, *et al.* Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J* 2014; **460:** 127–139.
82. Koyano F, Okatsu K, Kosako H, *et al.* Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 2014; **510:** 162–166.
83. Tanaka A, Cleland MM, Xu S, *et al.* Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 2010; **191:** 1367–1380.
84. Wang X, Winter D, Ashrafi G, *et al.* PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 2011; **147:** 893–906.
85. Lazarou M, Sliter DA, Kane LA, *et al.* The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 2015; **524:** 309–314.
86. Heo JM, Ordureau A, Paulo JA, *et al.* The PINK1–PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. *Mol Cell* 2015; **60:** 7–20.
87. Richter B, Sliter DA, Herhaus L, *et al.* Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. *Proc Natl Acad Sci U S A* 2016; **113:** 4039–4044.
88. McLelland GL, Soubannier V, Chen CX, *et al.* Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J* 2014; **33:** 282–295.
89. Bingol B, Tea JS, Phu L, *et al.* The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* 2014; **510:** 370–375.
90. Rakovic A, Grunewald A, Kottwitz J, *et al.* Mutations in PINK1 and Parkin impair ubiquitination of Mitofusins in human fibroblasts. *PLoS One* 2011; **6:** e16746.
91. Seibler P, Graziotto J, Jeong H, *et al.* Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. *J Neurosci* 2011; **31:** 5970–5976.
92. Michiorri S, Gelmetti V, Giarda E, *et al.* The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy. *Cell Death Differ* 2010; **17:** 962–974.
93. Hamasaki M, Furuta N, Matsuda A, *et al.* Autophagosomes form at ER–mitochondria contact sites. *Nature* 2013; **495:** 389–393.
94. Hertz NT, Berthet A, Sos ML, *et al.* A neo-substrate that amplifies catalytic activity of Parkinson's-disease-related kinase PINK1. *Cell* 2013; **154:** 737–747.
95. Cali T, Ottolini D, Brini M. Mitochondrial Ca^{2+} and neurodegeneration. *Cell Calcium* 2012; **52:** 73–85.
96. Gandhi S, Wood-Kaczmar A, Yao Z, *et al.* PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell* 2009; **33:** 627–638.
97. Rutter GA, Rizzuto R. Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection. *Trends Biochem Sci* 2000; **25:** 215–221.
98. Marongiu R, Spencer B, Crews L, *et al.* Mutant Pink1 induces mitochondrial dysfunction in a neuronal cell model of Parkinson's disease by disturbing calcium flux. *J Neurochem* 2009; **108:** 1561–1574.
99. Patergnani S, Suski JM, Agnoletto C, *et al.* Calcium signaling around mitochondria associated membranes (MAMs). *Cell Commun Signal* 2011; **9:** 19.
100. Schapira AH, Cooper JM, Dexter D, *et al.* Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1989; **1:** 1269.
101. Gomez C, Bandez MJ, Navarro A. Pesticides and impairment of mitochondrial function in relation with the parkinsonian syndrome. *Front Biosci* 2007; **12:** 1079–1093.
102. Gautier CA, Giaime E, Caballero E, *et al.* Regulation of mitochondrial permeability transition pore by PINK1. *Mol Neurodegener* 2012; **7:** 22.
103. Amo T, Sato S, Saiki S, *et al.* Mitochondrial membrane potential decrease caused by loss of PINK1 is not due to proton leak, but to respiratory chain defects. *Neurobiol Dis* 2011; **41:** 111–118.
104. Vos M, Esposito G, Edirisinghe JN, *et al.* Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. *Science* 2012; **336:** 1306–1310.
105. Hoepken HH, Gispert S, Morales B, *et al.* Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. *Neurobiol Dis* 2007; **25:** 401–411.
106. Gautier CA, Kitada T, Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proc Natl Acad Sci U S A* 2008; **105:** 11364–11369.
107. Grunewald A, Gegg ME, Taanman JW, *et al.* Differential effects of PINK1 nonsense and missense mutations on mitochondrial function and morphology. *Exp Neurol* 2009; **219:** 266–273.
108. Abramov AY, Gegg M, Grunewald A, *et al.* Bioenergetic consequences of PINK1 mutations in Parkinson disease. *PLoS One* 2011; **6:** e25622.
109. Liu W, Acin-Perez R, Geghman KD, *et al.* Pink1 regulates the oxidative phosphorylation machinery via mitochondrial fission. *Proc Natl Acad Sci U S A* 2011; **108:** 12920–12924.
110. Vilain S, Esposito G, Haddad D, *et al.* The yeast complex I equivalent NADH dehydrogenase rescues pink1 mutants. *PLoS Genet* 2012; **8:** e1002456.
111. Morais VA, Haddad D, Craessaerts K, *et al.* PINK1 loss-of-function mutations affect mitochondrial complex I activity via NdufA10 ubiquinone uncoupling. *Science* 2014; **344:** 203–207.
112. Gehrke S, Wu Z, Klinkenberg M, *et al.* PINK1 and Parkin control localized translation of respiratory chain component mRNAs on mitochondria outer membrane. *Cell Metab* 2015; **21:** 95–108.
113. Costa AC, Loh SH, Martins LM. *Drosophila* Trap1 protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease. *Cell Death Dis* 2013; **4:** e467.
114. Plun-Favreau H, Klupsch K, Moisoi N, *et al.* The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nature Cell Biol* 2007; **9:** 1243–1252.
115. Moisoi N, Klupsch K, Fedele V, *et al.* Mitochondrial dysfunction triggered by loss of HtrA2 results in the activation of a brain-specific transcriptional stress response. *Cell Death Differ* 2009; **16:** 449–464.
116. Steer EK, Dail MK, Chu CT. Beyond mitophagy: cytosolic PINK1 as a messenger of mitochondrial health. *Antioxid Redox Signal* 2015; **22:** 1047–1059.
117. Dagda RK, Pien I, Wang R, *et al.* Beyond the mitochondrion: cytosolic PINK1 remodels dendrites through protein kinase A. *J Neurochem* 2014; **128:** 864–877.
118. Dickey AS, Strack S. PKA/AKAP1 and PP2A/Bβ2 regulate neuronal morphogenesis via Drp1 phosphorylation and mitochondrial bioenergetics. *J Neurosci* 2011; **31:** 15716–15726.
119. Ries V, Henchcliffe C, Kareva T, *et al.* Oncoprotein Akt/PKB induces trophic effects in murine models of Parkinson's disease. *Proc Natl Acad Sci U S A* 2006; **103:** 18757–18762.
120. Dagda RK, Cherra SJ 3rd, Kulich SM, *et al.* Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *J Biol Chem* 2009; **284:** 13843–13855.
121. Fedorowicz MA, de Vries-Schneider RL, Rub C, *et al.* Cytosolic cleaved PINK1 represses Parkin translocation to mitochondria and mitophagy. *EMBO Rep* 2014; **15:** 86–93.
122. Xiong H, Wang D, Chen L, *et al.* Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *J Clin Invest* 2009; **119:** 650–660.

123. Nakajima A, Kataoka K, Hong M, *et al.* BRPK, a novel protein kinase showing increased expression in mouse cancer cell lines with higher metastatic potential. *Cancer Lett* 2003; **201:** 195–201.
124. Inzelberg R, Jankovic J. Are Parkinson disease patients protected from some but not all cancers? *Neurology* 2007; **69:** 1542–1550.
125. MacKeigan JP, Murphy LO, Blenis J. Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nature Cell Biol* 2005; **7:** 591–600.
126. Martin SA, Hewish M, Sims D, *et al.* Parallel high-throughput RNA interference screens identify PINK1 as a potential therapeutic target for the treatment of DNA mismatch repair-deficient cancers. *Cancer Res* 2011; **71:** 1836–1848.
127. Jin Y, Murata H, Sakaguchi M, *et al.* Partial sensitization of human bladder cancer cells to a gene-therapeutic adenovirus carrying REIC/Dkk-3 by downregulation of BRPK/PINK1. *Oncol Rep* 2012; **27:** 695–699.
128. Willis S, Villalobos VM, Gevaert O, *et al.* Single gene prognostic biomarkers in ovarian cancer: a meta-analysis. *PLoS One* 2016; **11:** e0149183.
129. O'Flanagan CH, Morais VA, Wurst W, *et al.* The Parkinson's gene *PINK1* regulates cell cycle progression and promotes cancer-associated phenotypes. *Oncogene* 2015; **34:** 1363–1374.
130. Taguchi N, Ishihara N, Jofuku A, *et al.* Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem* 2007; **282:** 11521–11529.
131. Grigoreva TA, Tribulovich VG, Garabadzhiu AV, *et al.* The 26S proteasome is a multifaceted target for anti-cancer therapies. *Oncotarget* 2015; **6:** 24733–24749.
132. Gomes LR, Vessoni AT, Menck CF. Microenvironment and autophagy cross-talk: implications in cancer therapy. *Pharmacol Res* 2016; **107:** 300–307.
133. Chourasia AH, Boland ML, Macleod KF. Mitophagy and cancer. *Cancer Metab* 2015; **3:** 4.
134. Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. *Nature Chem Biol* 2015; **11:** 9–15.
135. Heeman B, Van den Haute C, Aelvoet SA, *et al.* Depletion of PINK1 affects mitochondrial metabolism, calcium homeostasis and energy maintenance. *J Cell Sci* 2011; **124:** 1115–1125.
136. Piccoli C, Sardanelli A, Scrima R, *et al.* Mitochondrial respiratory dysfunction in familiar parkinsonism associated with PINK1 mutation. *Neurochem Res* 2008; **33:** 2565–2574.
137. Yao Z, Gandhi S, Burchell VS, *et al.* Cell metabolism affects selective vulnerability in PINK1-associated Parkinson's disease. *J Cell Sci* 2011; **124:** 4194–4202.
138. Akundi RS, Zhi L, Sullivan PG, *et al.* Shared and cell type-specific mitochondrial defects and metabolic adaptations in primary cells from PINK1-deficient mice. *Neurodegener Dis* 2013; **12:** 136–149.
139. Requejo-Aguilar R, Lopez-Fabuel I, Fernandez E, *et al.* PINK1 deficiency sustains cell proliferation by reprogramming glucose metabolism through HIF1. *Nature Commun* 2014; **5:** 4514.
140. Tufi R, Gandhi S, de Castro IP, *et al.* Enhancing nucleotide metabolism protects against mitochondrial dysfunction and neurodegeneration in a PINK1 model of Parkinson's disease. *Nature Cell Biol* 2014; **16:** 157–166.
141. Bagchi A, Mills AA. The quest for the 1p36 tumor suppressor. *Cancer Res* 2008; **68:** 2551–2556.
142. Tan JM, Dawson TM. Parkin blushed by PINK1. *Neuron* 2006; **50:** 527–529.
143. Agnihotri S, Golbourn B, Huang X, *et al.* PINK1 is a negative regulator of growth and the Warburg effect in glioblastoma. *Cancer Res* 2016; **76:** 4708–4719.
144. Pugh TJ, Morozova O, Attiyeh EF, *et al.* The genetic landscape of high-risk neuroblastoma. *Nature Genet* 2013; **45:** 279–284.
145. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol* 2014; **24:** R453–R462.
146. Putti R, Migliaccio V, Sica R, *et al.* Skeletal muscle mitochondrial bioenergetics and morphology in high fat diet induced obesity and insulin resistance: focus on dietary fat source. *Front Physiol* 2015; **6:** 426.
147. Scheele C, Nielsen AR, Walden TB, *et al.* Altered regulation of the PINK1 locus: a link between type 2 diabetes and neurodegeneration? *FASEB J* 2007; **21:** 3653–3665.
148. Xu X, Kobayashi S, Chen K, *et al.* Diminished autophagy limits cardiac injury in mouse models of type 1 diabetes. *J Biol Chem* 2013; **288:** 18077–18092.
149. Choi J, Ravipati A, Nimmagadda V, *et al.* Potential roles of PINK1 for increased PGC-1α-mediated mitochondrial fatty acid oxidation and their associations with Alzheimer disease and diabetes. *Mitochondrion* 2014; **18:** 41–48.
150. Franks PW, Scheele C, Loos RJ, *et al.* Genomic variants at the *PINK1* locus are associated with transcript abundance and plasma nonesterified fatty acid concentrations in European whites. *FASEB J* 2008; **22:** 3135–3145.
151. Deas E, Piipari K, Machhada A, *et al.* PINK1 deficiency in β-cells increases basal insulin secretion and improves glucose tolerance in mice. *Open Biol* 2014; **4:** 140051.
152. Zhang J, Chen K, Wang L, *et al.* Phosphatase and tensin homologue (PTEN)-induced putative kinase 1 reduces pancreatic beta-cells apoptosis in glucotoxicity through activation of autophagy. *Biochem Biophys Res Commun* 2016; **476:** 299–305.
153. Cummins TD, Holden CR, Sansbury BE, *et al.* Metabolic remodeling of white adipose tissue in obesity. *Am J Physiol Endocrinol Metab* 2014; **307:** E262–E277.
154. Dorn GW 2nd. Parkin-dependent mitophagy in the heart. *J Mol Cell Cardiol* 2016; **95:** 42–49.
155. Billia F, Hauck L, Konecny F, *et al.* PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci U S A* 2011; **108:** 9572–9577.
156. Siddall HK, Yellon DM, Ong SB, *et al.* Loss of PINK1 increases the heart's vulnerability to ischemia–reperfusion injury. *PLoS One* 2013; **8:** e62400.
157. Roe ND, Xu X, Kandadi MR, *et al.* Targeted deletion of PTEN in cardiomyocytes renders cardiac contractile dysfunction through interruption of Pink1–AMPK signaling and autophagy. *Biochim Biophys Acta* 2015; **1852:** 290–298.
158. Sengupta A, Molkentin JD, Paik JH, *et al.* FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress. *J Biol Chem* 2011; **286:** 7468–7478.
159. Wang K, Zhou LY, Wang JX, *et al.* E2F1-dependent miR-421 regulates mitochondrial fragmentation and myocardial infarction by targeting Pink1. *Nature Commun* 2015; **6:** 7619.
160. Gong G, Song M, Csordas G, *et al.* Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science* 2015; **350:** aad2459. DOI: 10.1126/science.aad2459.
161. Hoffmann RF, Zarrintan S, Brandenburg SM, *et al.* Prolonged cigarette smoke exposure alters mitochondrial structure and function in airway epithelial cells. *Respir Res* 2013; **14:** 97.
162. Mizumura K, Cloonan SM, Nakahira K, *et al.* Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *J Clin Invest* 2014; **124:** 3987–4003.
163. Ahmad T, Sundar IK, Lerner CA, *et al.* Impaired mitophagy leads to cigarette smoke stress-induced cellular senescence: implications for chronic obstructive pulmonary disease. *FASEB J* 2015; **29:** 2912–2929.
164. Ito S, Araya J, Kurita Y, *et al.* PARK2-mediated mitophagy is involved in regulation of HBEC senescence in COPD pathogenesis. *Autophagy* 2015; **11:** 547–559.

165. Patel AS, Song JW, Chu SG, *et al.* Epithelial cell mitochondrial dysfunction and PINK1 are induced by transforming growth factor-β1 in pulmonary fibrosis. *PLoS One* 2015; **10:** e0121246.
166. Bueno M, Lai YC, Romero Y, *et al.* PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J Clin Invest* 2015; **125:** 521–538.
167. Sosulski ML, Gongora R, Danchuk S, *et al.* Deregulation of selective autophagy during aging and pulmonary fibrosis: the role of TGFβ1. *Aging Cell* 2015; **14:** 774–783.
168. Stanaway JD, Flaxman AD, Naghavi M, *et al.* The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet* 2016; **388:** 1081–1088.
169. Kim SJ, Khan M, Quan J, *et al.* Hepatitis B virus disrupts mitochondrial dynamics: induces fission and mitophagy to attenuate apoptosis. *PLoS Pathog* 2013; **9:** e1003722.
170. Kim SJ, Syed GH, Siddiqui A. Hepatitis C virus induces the mitochondrial translocation of Parkin and subsequent mitophagy. *PLoS Pathog* 2013; **9:** e1003285.
171. Gelmetti V, De Rosa P, Torosantucci L, *et al.* PINK1 and Beclin1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation. *Autophagy* 2016 (in press).