MULTIPLE CELLULAR FUNCTIONS OF PINK1, A KEY MITOCHONDRIAL KINASE IN PARKINSON'S DISEASE

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Abstract. Parkinson's disease (PD) is the second most common neurodegenerative condition associated with aging. The disease advances over many years and presents progressive cognitive and motor function disturbances, which can lead to incapacity to perform independently daily activities. Environmental factors, aging and genetic susceptibility are recognized factors that contribute to the complex etiology of Parkinson's disease. Studying genetic models of PD supports the quest to understand causes and mechanisms of neurodegeneration in PD and points toward novel pharmaceutical approaches to tackle the disease progression. Loss-of-function mutations in the mitochondrial protein kinase PINK1 are causative in early onset autosomal recessive Parkinson's disease. PINK1 is involved in multiple cellular functions including mitochondrial quality control, calcium homeostasis, cell death and survival mechanisms. Here we review key roles of PINK1 in mitochondrial and nonmitochondrial function and emphasize its implication in the etiopathogenesis of PD in comparison to other diseases.

Key words: Parkinson's disease, PINK1, NCLX, pace-marker, dopaminergic neurons, heart.

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders occurring with high prevalence in aging. It affects about 1–2% of the population over 65 years and 4% of the people over 85 years old [48]. The characteristic neuropathologic markers of the disease are loss of dopaminergic neurons from substantia nigra (SN) and aggregation of misfolded proteins in structures known as Lewy bodies and Lewy neurites. These aggregates contain primarily alpha-synuclein and ubiquitin together with other biochemical markers [12]. These pathogenic structures are also present in other regions of the brain such

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as the dorsal motor nucleus of the vagus, locus coeruleus, thalamus, amygdalae, olfactory nuclei, and cerebral cortex [55]. The pathogenesis of protein aggregation in PD has a characteristic spreading pattern that associates with the disease progression, the so-called 'Braak staging of PD'. The first areas affected are the dorsal motor nucleus of the glossopharyngeal, the vagal nerves, and the anterior olfactory bulb [8]. Correlated with the function of the damaged brain areas, the most common symptoms affect motor functions including difficulty of movement, rigidity, postural instability, and tremor. During the progression of the disease the patients may also suffer from non-motor symptoms like anxiety, depression, sleep disturbances, impairment of cognitive functions and dementia [66]. Etiology of Parkinson's includes environmental factors, genetic susceptibility and aging. Although the molecular mechanisms that characterize PD are still unclear, oxidative stress and mitochondrial dysfunctions are known as important factors that trigger neuronal death and are exacerbated in parkinsonism [71]. Discovery of toxins, like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, paraquat, rotenone, 6-hydroxydopamine that induce Parkinson's disease, and are used in generating PD animal models, support the importance of environmental factors in the etiology of the disease [55]. These toxins are implicated in PD pathogenesis through mechanisms of oxidative stress and mitochondrial dysfunction. Besides the environmental risk, it is now well established that many forms of PD involve genetic susceptibility factors [58]. Most known genes that contribute to Parkinson's encode alpha-synuclein (SNCA gene), Leucine-rich repeat kinase 2 (LRRK2) (LRRK2, PARK8 gene), Parkin (PARK2 gene), PINK1 (PARK6 gene), DJ-1 (PARK7 gene). The first two of the mentioned genes are autosomal dominant, the others being autosomal recessive [48]. Mutations of SNCA are rare and generate abundant inclusions of alfa-synuclein in neurons that lead either to classic PD, Lewy body disease or multiple system atrophy. LRRK2 encode a very large protein with multiple functional domains. LRRK2 mutations represent the most common genetic factor involved in etiology of PD, the clinical features being similar to classic idiopathic PD. Parkin mutations represent the most common cause among the autosomal recessive forms of genetic PD followed by mutations in PINK1 and DJ-1. None of these PD forms present atypical clinical characteristics [58].

PINK1: A MITOCHONDRIAL PROTEIN KINASE WITH MULTIPLE FUNCTIONS

Mitochondria are essential organelles for eukaryotic cells, being involved in key functions such as energy production (ATP), apoptosis, redox processes, calcium homeostasis and metabolism, their dysfunction being associated with various diseases including cancer, diabetes and neurodegeneration [11]. Mitochondria process high amounts of oxygen during the oxidative phosporylation process of the ATP production cycle, generating reactive oxygen species (ROS) as

a by-product. ROS can produce cellular damage at multiple levels affecting the DNA, proteins, lipids and carbohydrates. To avoid these detrimental effects they have achieved quality control mechanisms that act at the molecular or organellar level. The molecular quality control mechanism involves the recognition of damaged components by sensor molecules that signal upregulation of molecules able to repair or eliminate the lesion. When more extended damages occur, the organellar quality control mechanisms are activated, leading to fusion, fission or autophagy of the impaired mitochondria [17].

PINK 1 (phosphatase tensin-homolog (PTEN) induced kinase 1) represents a ubiquitous mitochondrial serine-threonine kinase, highly expressed in the heart, skeletal muscle, testes and brain (with a higher expression in substantia nigra, hippocampus and Purkinje cells) [22]. PINK1 is involved in several mitochondrial processes that are tightly connected to one another and play important roles in cellular function. These include calcium homeostasis [57], mitochondrial respiration [23], ATP production [35], ROS formation [28]; apoptosis and mitochondria quality control [39].

PINK1: STRUCTURE AND LOCALIZATION

PINK1 is encoded by an autosomal gene, PARK6, from the chromosomal locus 1p35-36 that contains 8 exones [27]. PINK1 is a highly conserved kinase, being 75–85% similar between mammalian orthologs [53] (Fig. 1). Human PINK1 contains 581 amino acids. The N-terminal region of the PINK1 precursor is involved in the mitochondrial localization at the inner mitochondrial membrane, which is dependent on the mitochondrial membrane potential integrity, but is not necessary for its processing [3]. The protein contains a transmembrane helix, a kinase domain (112–496) [62], and a C-terminal regulatory sequence involved in the retention of PINK1 at the outer mitochondrial membrane, in the case of membrane depolarization [3]. The protein is proteolytically processed first by matrix processing peptidase (MPP) and then by presenilin-associated rhomboid-like protein (PARL) to produce two N-terminally truncated protein fragments of 54 and 45 kDa without mitochondrial localization sequence. Following these cleavages truncated PINK1 products exit the mitochondria and localize in the cytosol where they are degraded by the proteosome. In the cytosol PINK1 binds to Parkin, blocking its translocation to mitochondria and preventing mitophagy [20]. The crystal structure of PINK1 has not been studied so far, but the homology with another serine-threonine kinase indicates a good similarity with members of the calmodulin-kinase family [10]. These enzymes mediate many intracellular responses to elevated calcium concentration, being a family of protein kinases whose activity is modulated by calcium/calmodulin binding and by phosphorylation [64]. These enzymes contain the catalytic site and the ATP/Mg^{2+} binding region between the two loops of the N-terminal and C-terminal [10].

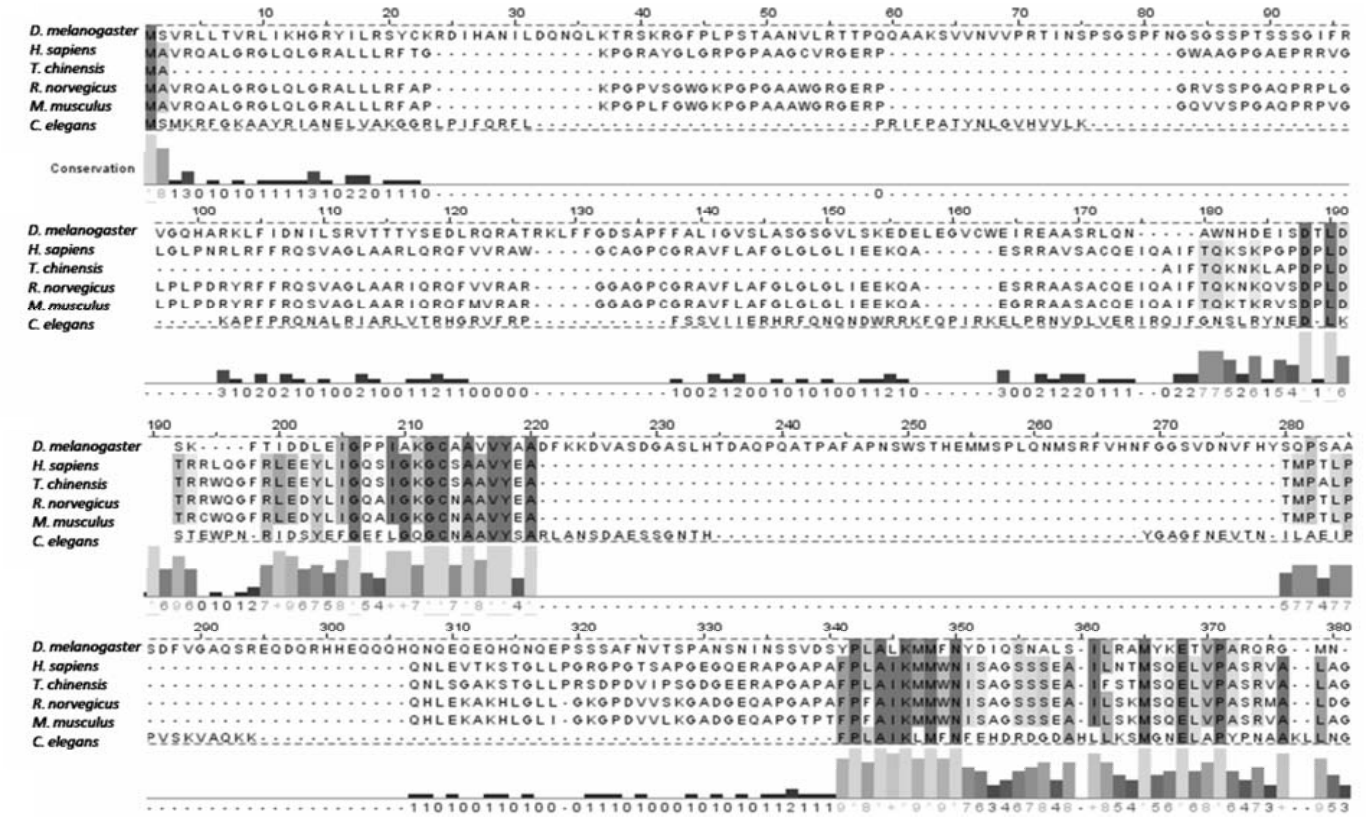

Fig. 1. Alignment of PINK1 protein sequences from different species: *Drosophila melanogaster* (AAN09178.1), *Homo sapiens* (NP_115785.1), *Tupaia chinensis* (ELW68528.1), *Rattus norvegicus* (AAI69047.1), *Mus musculus* (NP_081156.2), *Caenorhabidis elegans* (NP_495017.1). The regions highlighted in blue are areas of high degree of homology between these species. The alignment was done using JalView software (http://www.jalview.org/).

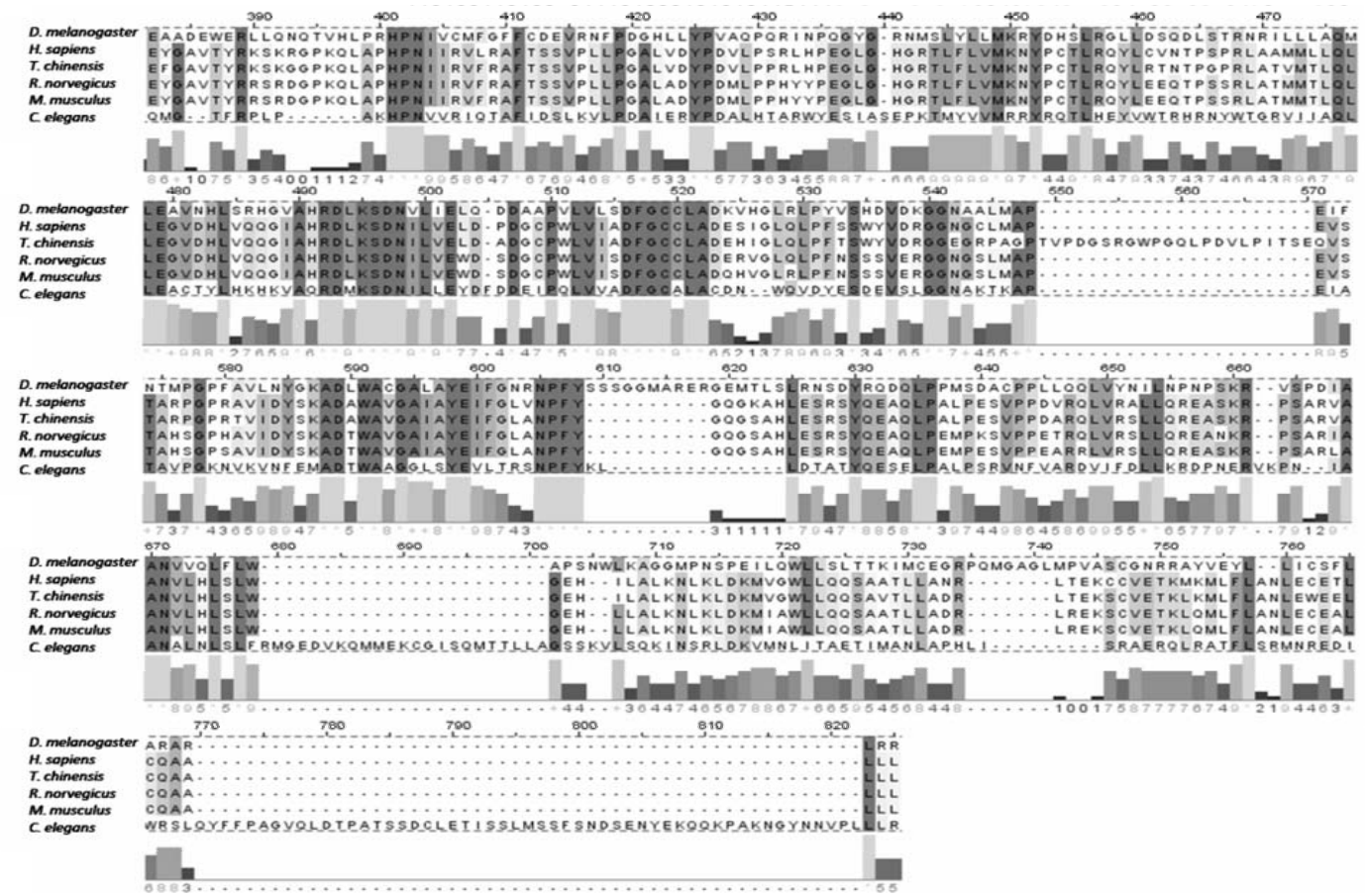

Fig. 1. (continued).

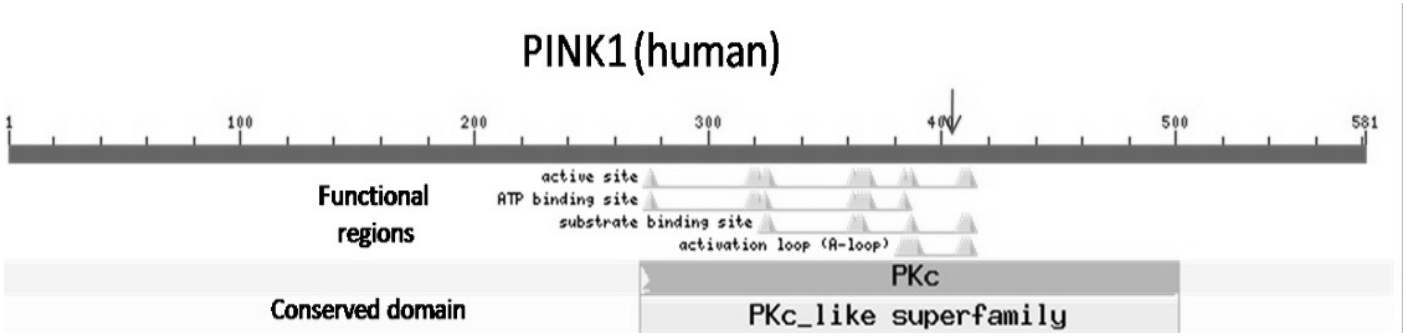

Fig. 2. Schematic representation of human PINK1 functional domains. The protein contains a protein-kinase catalytic domain (NCBI Protein Database) with two serine residues, marked here with an arrow, important for kinase activity.

PINK1 exposes a loop in the N-terminal region, involved in its mitochondrial localization. The subcellular and mitochondrial localization of PINK1 was debated for a long time. It is now accepted that the protein is embedded in the external membrane of the mitochondria, recent studies showing that the kinase domain faces the cytoplasm [68]. PINK1 contain a kinase and regulatory domains in the C-terminal domain. The three-dimensional structure of the protein is similar to other Ser/Thr kinases such as α-kinases or PKA, enzymes that contain three loops: phosphate binding (P loop), a catalytic loop and an activation loop [65]. The kinase domain of PINK1 consists of a loop containing two serine residues (Ser401 and Ser 402), similar to other kinases; phosphorylation of these amino acids leads to activation of the kinase function (Fig. 2). Another important amino acid is Arg407 that seems to interact with the serine residues, after their phosophorylation thus contributing to modulation of the kinase activity [47].

Several mutations of the gene have been associated with early onset Parkinson's disease. Most of them are loss of function mutations that affect the kinase domain of the protein [54].

PINK1: MITOCHONDRIAL FUNCTIONS

Correlated with its essential roles, PINK1 loss-of-function induces drastic functional and structural dysfunction in mitochondria. This has been established in *Drosophila melanogaster* studies where the PINK1 mutants present fragmented cristae, loss of outer membrane integrity, increased sensitivity to ROS, decreased ATP production, motor behavior impairments, and shorter live span [50, 13]. Moreover the mobilization of reserve pool synaptic vesicles at the neuromuscular junction of PINK1 deficient flies is impaired during rapid stimulation due to synaptic ATP depletion, indicating that synaptic activity cannot be maintained under increased energy demand in PINK1 deficient neurons [43].

In mammalian models of PD based on PINK1 loss-of-function there is higher variability in the data provided by the literature due to the differences in the models and experimental methodologies (Fig. 3).

Deletion of PINK1 in mice does not result in an overt phenotype. The mice display only subtle deficits, which differ slightly between different loss-of-function models, but converge to give a whole picture of a prodromal model of PD. Thus the phenotype presents only a minor decrease in total dopamine levels in very old mice [24], impaired synaptic plasticity in the striatum, but no loss of dopaminergic neurons in substantia nigra [33] respectively a minor loss of dopamine cells in a more recent conditional model of PINK1 loss-of-function [25]. Mitochondrial respiration is impaired in the striatum of PINK1 KO and respiration deficits can be induced in the cortex by cellular stress [23]. In a new mouse model of PD, PINK1 loss-of-function accelerates *in vivo* neurodegenerative phenotypes induced by accumulation of unfolded proteins in the mitochondria and consequent mitochondrial dysfunction [42].

Mitochondrial morphology

Although PINK1 defects induce subtle morphological and functional changes in the mitochondria, none of the studies developed in mammalian systems with PINK1 loss-of-function cells displayed the dramatic phenotypes described in Drosophila [23, 28]. Thus, in primary striatal culture derived from mice, the average number and size of mitochondria were the same in wild type mice or PINK1 KO mutants, with an increased percentage of enlarged organelles in the KO cells [23] while in mouse embryonic fibroblasts (MEFs) PINK1 depletion caused moderate mitochondrial fragmentation [28]. Another study in neuroblastoma demonstrated that PINK1 deficiency is correlated with mitochondrial fragmentation while its overexpression induced elongated, interconnected mitochondria [15]. These suggest that PINK1 has a pro-fusion role in mammalian cells, but affects differently the balance between fusion and fission, according to the cell type. Reduced fission can lead to a reduced ability of the cell to remove unfunctional mitochondria by mitophagy [56].

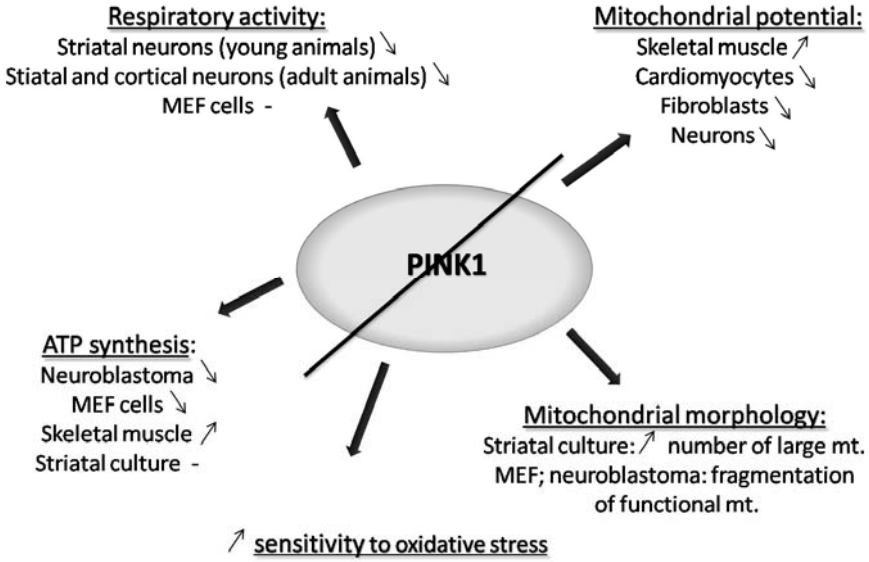

Fig. 3. PINK1 loss-of-function affects multiple cell type specific mitochondrial functions.

These processes involve GTPase activity, enzyme that seems to be differently expressed in different cell types, correlated to the bioenergetics demands. An important GTPase in mitochondrial fusion is mitofusin (Mfn) Molecules from distinct mitochondria interact and allow fusion of the outer mitochondrial membranes, followed by fusion of the internal membrane by optic atrophy 1 (Opa1) interaction. Mfn ubiquitination by PINK1 or Parkin accelerate proteosomal

degradation. It also interacts with Miro, a protein involved in axonal and dendridic trafficking of mitochondria, in order to control mitochondrial transport. PINK1 phosphorylates Mfn2 that will bind Parkin at the mitochondria being involved in mitophagy. Fission process is driven by accumulation of Dynamin-related protein 1 (Drp1) on mitochondrial membrane, allowing the organelle to undergo fission [56].

Mitophagy

In healthy mitochondria with a normal membrane potential, PINK1 is degraded by proteolysis [26]; following depolarization of the inner membrane, the protein accumulates on the mitochondrial surface and becomes active [45]. In this state PINK1 phosphorylates the E3-ubiquitin ligase Parkin at Ser65, within an Ubiquitin (Ub) like domain, an essential step required for Parkin's activation and translocation to mitochondria. Other PINK1 substrates also activate Parkin. The kinase can phosphorylate Ub at Ser65, subsequently activating Parkin [31, 30, 34]. Expression of a mutant form of Ub that cannot be phosphorylated by PINK1, suppresses Parkin translocation to mitochondria [30]. In mitochondria, the phosphorylated Parkin is implicated in removal of dysfunctional organelles [14] by ubiquitylation of substrates on the outer mitochondrial membrane like voltage-dependent anion channel (VDAC1) and mitofusin [70]. Interestingly, in PINK1 deficient cells Parkin expression was increased in what was hypothesised to comprise a compensatory mechanism [14] together with an amplification of autophagy particularly mitophagy processes [15].

Respiratory activity

Analysis of mitochondrial function revealed that loss of PINK1 induces a decrease in the activity of respiratory complexes specific to striatal neurons for young animals, while in older mice the decrease was also significant in cortical neurons [23]. Studies on MEF revealed normal respiratory activity in PINK1 deficient cells [28]. The level of oxidative stress was not significantly different between wild type and PINK1 KO mice, but the mutants were more sensitive to exogenous stressors such as H_2O_2 or heat shock, that exacerbate the respiratory defects [23]; in a study on MEF cells, PINK1 KO or KD had increased level of ROS compared to MEF wild type [28].

ATP synthesis

PINK1 deficient MEF cells [28], and neuroblastoma [35] were found to have reduced ATP synthesis; this was not observed in the brain (striatum) of PINK1 deficient mice [23]. On the contrary, in PINK1 deficient skeletal muscular cells the ATP level was found to be increased [67].

Mitochondrial membrane potential

Different studies showed opposite results concerning changes in mitochondrial potential in PINK1 deficient cells, according to the cell type. In skeletal muscle myocytes the mitochondrial potential increased in PINK1 deficient cells [67], while in cardiomyocytes [57], fibroblasts, and neurons [67] the potential decreased. These differential effects point towards increased sensitivity of neuronal cells for PINK1-loss related mitochondrial dysfunction.

PINK1 AND MITOCHONDIRAL CALCIUM HOMEOSTASIS

A crucial mitochondrial function is maintenance of calcium homeostasis that regulates general mitochondrial and cellular mechanisms such as ATP production [7], cell death/survival [46], but also cell-type specific metabolic processes such as glutamate transmission [46] or automatism function [61]. Calcium ions enter the organelle by a uniport ion channel [32] and are re-delivered to the cytosol either by a protons-coupled exchanger in non-excitable cells [19], or by a sodium-calcium antiport exchanger for excitable cells [49]. Although the functional properties of the Na/Ca exchanger were studied for a long time, its molecular identity was only recently revealed. The exchanger is located in mitochondrial cristae inner membrane and can mediate Li/Ca as well as Na/Ca exchange, being named Na/Li/Ca exchanger (NCLX) [49].

Loss of PINK1 function impairs the mitochondrial calcium homeostasis leading to accumulation of the ions in the organelle. Studies of Gandhi (and collaborators) proved that in the absence of PINK1 there was no efflux of Ca^{2+} from mitochondria, and neither Na^{+} influx, while Ca^{2+} influx in PINK1 KD/KO cells was normal, suggesting that a dysfunction of NCLX, would be the cause of accumulation of calcium ions in the organelle [22], leading to defects in the processes controlled by mitochondrial calcium concentration (Fig. 4). Mitochondrial calcium is important in regulation of energy production, many of the proteins involved in this process being calcium sensitive, including dehydrogenases from the Krebs cycle that provide substrates for the electron transport chain [7]. Increased mitochondrial calcium concentration induces a rapid increase in energy production, as reported by Yan and his collaborators in skeletal muscle cells [66].

A high rate of oxidative phosphorylation, together with increased mitochondrial calcium concentration, would also be correlated with an increase in ROS production [28]. The high ROS and calcium amount will finally lead to dysfunction in mitochondrial respiration [22] as was observed in neurons from young PINK1 KO mice striatum and at an older age also in the cortical area [23] and will lead to reduced ATP level, as was observed in studies on MEF cell [28] or neuroblastoma [35]. These processes will contribute to impaired calcium efflux and induce depolarization of the inner membrane finally leading to open of mPTP [22].

The mPTP opening in the mitochondrial membrane can contribute to early stages of apoptosis through decrease of the inner membrane potential or to necrotic changes following oxidative stress or ischemia. In all cases mPTP opening is associated with mitochondrial calcium accumulation [21].

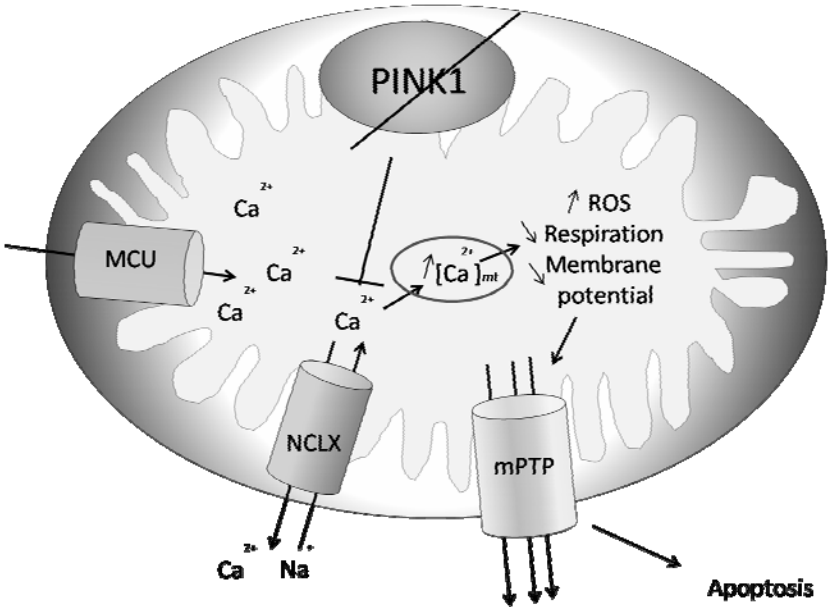

Fig. 4. PINK1 role in calcium homeostasis. PINK1 loss-of-function leads to impaired NCLX activity. This is consequently followed by accumulation of Ca^{2+} ions in mitochondria, resulting in generation of ROS, impaired oxidative phosphorylation, decreased membrane potential. These lead to mPTP opening and ultimately to induction of apoptosis.

PINK1 LOSS-OF-FUNCTION INCREASES VULNERABILITY OF CELLS WITH PACEMAKER FIRING ACTIVITY

Although some of the mechanisms by which PINK1 regulates key cellular functions are known, the processes responsible for specific loss of dopaminergic neurons related to PD are not fully elucidated. The neurons in SN seem to be particularly susceptible to mitochondrial dysfunction due to additional oxidative stress generated by dopamine metabolism [22]. In addition, it has been recently proposed that neurons with autonomous pacemaking function present increased sensitivity to mitochondrial dysfunction [59]. This property by which some neuronal cells generate regular spontaneous firing in the absence of synaptic input has been observed for dopaminergic neurons. Neurons from SN generate autonomous spinking, involving calcium entry through L-type Ca^{2+} channels from

the extracellular medium [52]; this autonomous firing is generated by a different mechanism as compared to other cells with pacemaking potentials from the central nervous system [60]. Deletion of PINK1 leads to a reduction in the activity of small-conductance Ca^{2+} activated K^{+} channels that induce irregular firing pattern in dopaminergic neurons but does not affect regular firing in GABAergic neurons from SN [6]. The frequent calcium influxes induce elevated mitochondrial stress, fact that may be a cause of the increased vulnerability of these cells to PINK1 loss-of-function.

A significant amount of information related to PINK1 function has arisen from studies performed in myocytes. These are also able to evoque autonomous pacemaker spikes, based partially on calcium entry from extracellular medium, together with the release of calcium from intracellular deposits and accompanied by regulated changes in levels of other ions [37]. Studying these model systems contributes to understanding the relationship between spontaneous membrane potentials and cell vulnerability to stress factors. Recent work on transgenic mice showed that loss of PINK1 increased the size of myocardial infarct following ischemia [57]. PINK1 deficiency also induced higher level of oxidative stress, increased cardiomyocyte apoptosis and fibrosis, contributed to ventricular dysfunction and cardiac hypertrophy [5]. *In vitro* experiments on cardiomyocytes obtained from PINK1 KO mice showed a lower resting mitochondrial potential, inhibited mitochondrial respiration [57] and increased sensitivity to ROS-dependent depolarization of the mitochondrial membrane [5]. Siddal and his collaborators worked on HL-1 cardiomyocytes, a cell line with spontaneous contractions, proving that over-expression PINK1 reduced cell death after ischemia and decreased susceptibility to mPTP opening [69]. NCLX knockdown on HL-1 cells alters cardiac automatism leading to prolonged cycle of calcium oscillations and action potential spikes [61].

In humans, patients with Parkinson's disease, present a two fold increase in risk for heart failure [69], a condition that represents the leading cause of morbidity and mortality in North America [9]. Recent studies showed that in end-stage heart failure patients the expression of PINK1 protein is decreased in ventricular tissue samples, irrespective of the etiology of their condition. However, the mRNA level was not different, suggesting a regulatory mechanism that acts at post-transcriptional level [5].

These findings sustain a correlation between autonomous pacemaker functions and increased vulnerability of PINK1 deficient cells that would be particularly important in the case of neurons that are usually not renewed during lifetime. Therefore, a better understanding of the molecular pathways regulating calcium homeostasis and how PINK1 is involved in these processes may provide new insights into the etiopathology of Parkinson's disease.

PINK1 AND CANCER

While loss of function mutations in PINK1 cause autosomal Parkinson's disease, increased PINK1 expression was found in different cancers, promoting cell survival and metastatic functions. PD patients present lower cancer rates with the exception of higher risk of malignant melanoma, skin, breast, and thyroid cancers [29]. The mechanisms underlying both these diseases are not completely deciphered but recent genetic and emerging functional studies point to similar and overlapping pathways [18]. Fig. 5 depicts the complexity of overlapping pathways in PD and cancer and shows how different cell processes (protein misfolding and degradation, cell cycle control and apoptosis, mitochondria and oxidative stress, the PI3K-AKT-mTOR pathway) may interact and have a pathogenic effect.

PINK1 was identified in HeLa cells as a gene upregulated by overexpression of the central tumor suppressor, PTEN [63]. The PTEN gene encodes a multifunctional phosphatase which plays an important role in inhibiting the PI3K/Akt pathway and mutations in PTEN have been found in many human cancers [16]. Recent studies indicate a role of PINK 1 in tumorigenesis and chemoresistance [1, 4, 36, 38].

The PINK1 function in cancer biology is far from being clear. Besides PTEN, PINK1 is also induced by other tumor suppressors like FOXO3a, Parkin (an autosomal recessive PD-triggering gene), Beclin-1 [40, 41]. Moreover, PINK1 mediated activation of AKT via mammalian mTOR complex increases migration which is a cardinal feature of cancer cells [44]. Recent work indicated that PINK1 is a cell cycle regulator and also a candidate oncogene. Thus deletion of PINK1 lead to cell cycle defects correlated with aberrant regulation of mitochondrial fission by Drp1, a critical step in progression of mitosis. PINK1 was also identified as a target for treatment of malignancies with DNA mismatch repair deficiency [38]. To add on the image of the complex role of PINK1 in biological processes it has also been postulated that PINK1 may have a tumor suppressive activity [2] or that PINK1 might have a dual role, acting either pro-survival or pro-death, depending on the biological context [4].

PINK1 protein appears to be a pleiotropic protein that is likely to have more than one role in mitochondria homeostasis, apoptosis, survival and proliferation processes. This results in PINK1 having distinct roles in different diseases. Moreover, PINK1 could undergo differential regulation depending on the particular biological characteristics of the process studied. Further work needs to be performed to understand the relative contributions of PINK1 to cancer and Parkinson's disease.

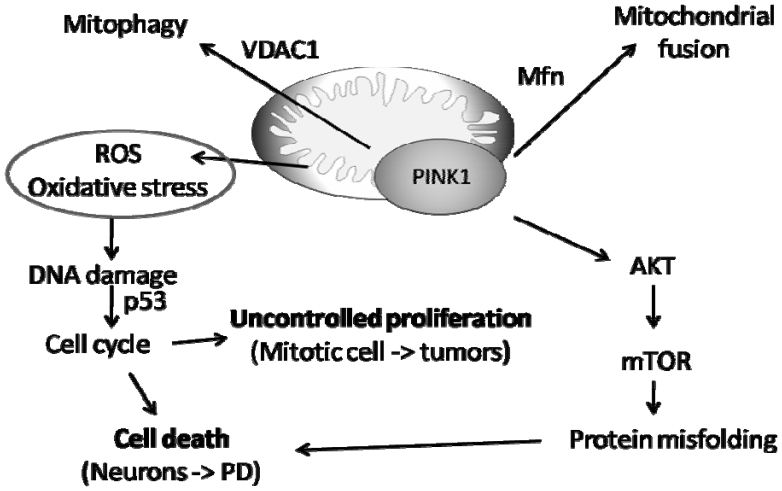

Fig. 5. PINK1 is involved in different cell mechanisms (mitochondrial fusion, by Mfn; mitophagy, through VDAC1; removal of misfolded protein, by AKT/mTOR pathway; generation of ROS/Oxidative stress) that finally control cell death or proliferation.

CONCLUDING REMARKS

PINK1 dysfunction induces changes in a multitude of cellular processes. The effects determined by PINK1 loss-of-function are dependent on the biological model investigated. However, the evidence converges to point towards a role for PINK1 in mitochondrial dysfunction through mitochondria quality control and mitochondrial metabolism. PINK1 mutations correlate with selective vulnerability of dopaminergic neurons from substantia nigra to stress, leading to development of Parkinson's disease.

PINK1 deficiency is accompanied by increased calcium levels in mitochondria, generated through NCLX dysfunction. Therefore, dysfunction in calcium homeostasis could lead to other detrimental effects at cellular level, and could be the reason of increased dopaminergic neurons vulnerability. It is known that these cells produce autonomous pacemaker potentials that occur with frequent increases in cytoplasmic and mitochondrial calcium levels making them more vulnerable to stress.

Given that genes linked to Parkinson’s disease have been considered potential oncogenes or tumor suppressors, further studies need to be done to understand the differential role of PINK1 in cancer and Parkinson's disease. The insights from one disease can inform us about the other, which could be important for development of future therapeutic strategies.

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