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Review Article

Parkinson disease: identifying different players sharing a common principle

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Parkinson disease is a multifactorial molecular disorder. Miscellaneous regulators have been characterized to date and their implication in disease progression is well documented. Compromised neuromuscular activity is a serious threat posed by malfunctioning of various regulators. The integrity and maintenance of neural architecture underpins neural activities. Despite the fact that various breakthroughs have been made, yet many proteins are unidentified while some unaddressed. Furthermore, miRNA pathway impairment results in subversion of core biological system and draws attention towards novel miRNA-based therapeutic strategies. Thus proteins and mitrons work in collaboration with various cellular organelles to ensure normal dynamics of neural circuitry. In this review we will emphasize the derailed activities of proteins at molecular level that might help in getting a step closer to personalized medicine.

Parkinson's disease is a neuro-degenerative disorder. There is an excessive loss of dopaminergic neurons. Recent research is on the brink of achieving a new level of understanding in terms of Parkinson's disease. Consistent milestones have been set in the comprehensions of this disorder. Neuronal death is the main mechanism that underlies the subversion of core biological system driving neuronal activities. In accordance with this assumption, delineation of the key players extensively implicated is necessary. A detailed mechanistic insight of the mitochondria and a better knowledge of the modulations engaged in apoptosis are competent for attention to bridge the existing gaps. In this review we will bring to limelight, the key proteins involved in disease progression, signal transduction cascades which drive neurodegeneration and new patterns in therapeutics with emphasis on miRNA.

Parkinson's disease

Parkinson's disease (PD), is statistically positioned to be the second most prevalent neurodegenerative disorder after Alzheimer's disease (AD) (Weintraub et al, 2008). James Parkinson being the first to explicate PD in 1817 in a publication "An Essay on the Shaking Palsy" (Parkinson, 1817). It is a slowly progressive neuro-degenerative molecular disorder with selective loss of dopaminergic neurons. Dopamine is a neurotransmitter, which is released into the synaptic cleft, in response to presynaptic action potential. It is noteworthy that dopamine controls voluntary movements, sleep, mood, working memory, learning etc. The common age of onset is in the early 60s, but up to 10% of affected are 45

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years or younger. Environmental and genetic factors are instrumental in the pathogenesis of PD but familial forms of PD are rare, just 5-10% (Weintraub *et al*., 2008; Lesage & Brice, 2009).

A typical feature seen in post-mortem brains of patients with PD is the presence of proteinaceous intracytoplasmic inclusions called Lewy bodies (LBs) (Lesage & Brice, 2009). The LBs are made up of numerous different proteins and their main component is α-synuclein and mitochondria (Wakabayashi *et al*., 2007). Many genes are associated with Parkinsonism. PINK1, Parkin, DJ-1 and HtrA2 are four of them (Lesage & Brice, 2009). PINK1, Park-6 is a mitochondrial kinase. This is responsible for early onset of PD (autosomal recessive and sporadic). Parkin is a ubiquitin E3 ligase also called Park-2 basis of EOPD (AR). A mitochondrial chaperon, DJ-1 also known as Park-7 also causes EOPD (AR) and Omi/HtrA2 a mitochondrial serine protease also called Park-13.

Structure of Mitochondria

Mitochondria play a vital role to maintain the integrity of neurons. Mitochondria have five sub compartments: outer mitochondrial membrane (OMM), intermembrane space (IMS), inner mitochondrial membrane (IMM), cristae and matrix. Cristae, are invaginations of the IMM, which increases the surface area of the IMM, enhancing its ability to produce ATP and separates proapoptotic proteins from matrix in the IMS (Zhang *et al*., 2008). The mtDNA is found in the matrix, the space enclosed by the IMM. mtDNA consists of a circular 16kb DNA molecule that encodes 37 genes. Several hundred copies of mtDNA can be found in one mitochondrion. I-IV complexes of the electron transport chain (ETC) and complex V, the ATPsynthase, which conducts ATP production, are embedded in the IMM. Mitochondria are ATP producers, particularly in neurons they are also responsible for Calcium signaling, protein degradation and apoptosis. Abnormal protein aggregation causes dysfunctioning, increased oxidative stress. This leads to the pathogenesis of PD (Zick *et al*., 2009).

Structure of PINK1 protein

In the human genome PINK1 also called PARK6.Located on the 1p36 locus. PINK1 gene has eight exons and encodes protein consist of 581 amino acids. N-terminal sequence, the Mitochondrial targeting sequence (MTS) proceeded by a transmembrane domain (TM), a Serine / threonine kinase domain being highly conserved (which resembles to the Ca2+/ calmodulin family of kinases) and at C-terminal a regulatory domain (Mills *et al.,* 2008; Silvestri *et al.,* 2005). Length of MTS is 1 - 77 amino acids. TM contains 94 – 110 amino acids. KD the highly conserved domain contains 156 – 511 amino acids and regulatory domain consists of 511 – 581 amino acids. The full-length of PINK1 protein is of ~63kDa. This is further proteolytically cleaved into ~55kDa by an unknown protease. In the mitochondria isolated from brain of rat, PINK1 protein is localized in both IMM and OMM (Gandhi *et al.,* 2006). Another study affirmed PINK1 in IMM and IMS (Gandhi *et al.,* 2006; Pridgeon *et al.,* 2007).

Fig 1: Structure of PINK1

PINK1 protects against oxidative-stress-induced cell death by phosphorylating mitochondrial TRAP1

A mutation in the PINK1 gene, which encodes a Serine/Threonine kinase leads to autosomal recessive form of Parkinson disease (PD) (Gegg *et al.,* 2009). Kinase domain of Pink1 is involved in the autophosphorylation and phosphorylation of mitochondrial substrate TRAP1 (tumor necrosis factor receptor–associated protein 1) respectively. Both PINK1 and TRAP1 are predicted to possess a mitochondria targeting signal at their N-terminal and both are localized in the mitochondrial intermembrane (Silvestri *et al.,* 2005). Fractionation studies on mitochondria show that PINK1 and TRAP1 both are found in the mitochondrial inner membrane and intermembrane space fractions (Gegg *et al.,* 2009).

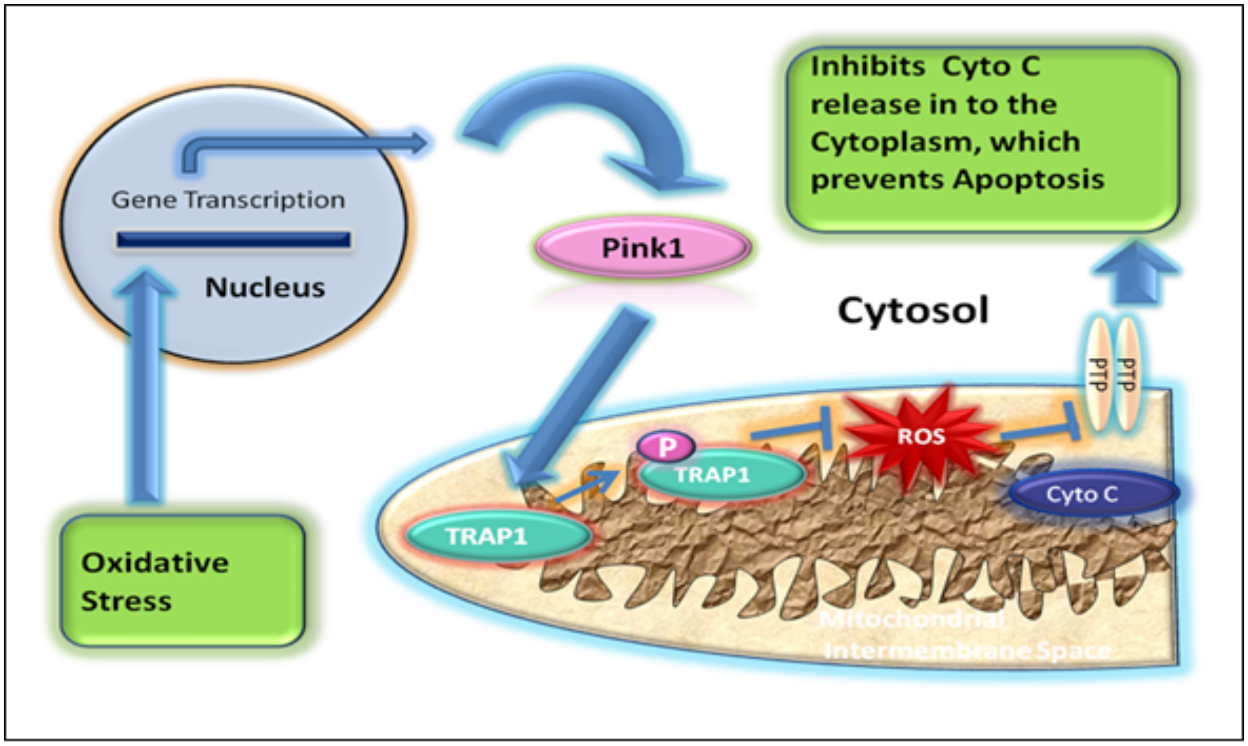

Figure 2: PINK1 protects cell from Oxidative Stress induced Apoptosis.

PINK1 mutations such as G309D, L347P, and W437X lead to impaired cell survival and its ability to phosphorylate TRAP1 (Pridgeon *et al.,* 2007; Kroemer and Blomgren, 2007). PINK1 kinase activity has not radically been affected by PCI2 cells with K219A mutation however the ability of PINK1 to phospohorylate TRAP1 is dramatically decreased during D362A, D384A and KDD triple mutations (Pridgeon *et al.,* 2007). TRAP1 is a mitochondrial heat shock protein 75 (Hsp75) and is chaperone in nature. TRAP1 and ROS act in opposition to each other i.e. if TRAP1 is silenced via RNA interference a significant increase in the accumulated ROS is observed (Hua *et al.,* 2007). Oxidative stress promptly resulted in an enhanced phosphorylation of TRAP1. The activity was remarkably increased in PINK1 overexpressed cell lines and there was a dramatic decline in the activity in PINK1 incompetent cells. This undercores the fact that TRAP1 is a direct substrate of PINK1.

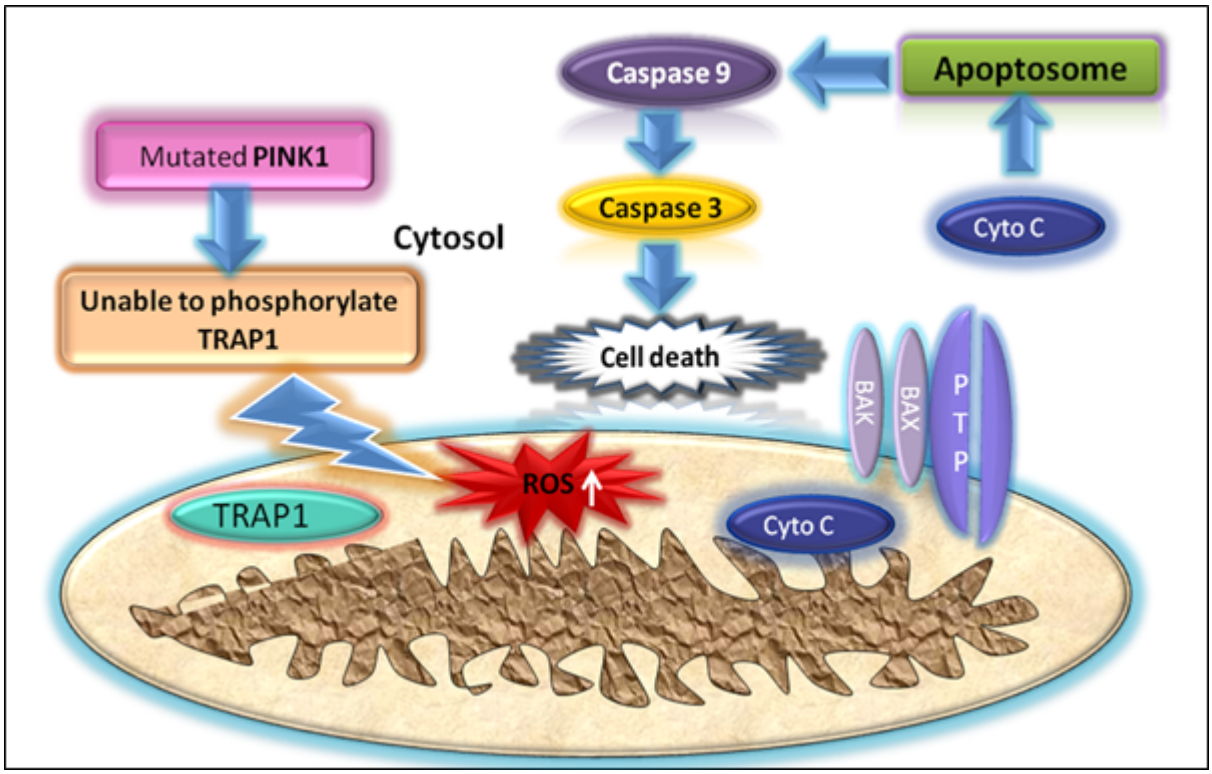

Figure 2.1: ROS induced dopaminergic neurons death due to mutated PINK1.

Phosphorylated TRAP1 then blocks the generation of mitochondrial ROS. This further blocks the opening of mitochondrial permeability transition pore (MPTP). It causes the inhibition of release of cytochrome c, smac and endonuclease G from mitochondria into cytosol (Xiang *et al.*, 2010). Phosphorylation of TRAP1 is significantly increased due to oxidative stress induced by H_2O_2. Over expression of wild-type PINK1 3-foldd increases the basal level of TRAP1 phosphorylation. PC12 Cells with wild-type PINK1 were much more resistant to H_2O_2-induced cell death as compared to PINK1 mutant cells (Pridgeon *et al.*, 2007). Pink1 indirectly protects dopaminergic neurons of substantia nigra from apoptosis induced cell death and promotes cell survival, thus it has a neuroprotective role in human brain (Kroemer and Blomgren, 2007; Pridgeon *et al.*, 2007). This evidence suggests that Trap1 and PINK1 are part of antiapoptotic signaling cascade (Tatsuta and Langer, 2008). TRAP1 chaperones the retinoblastoma protein. it regulates the cell-cycle. It is up regulated by the oncogene c-Myc and down-regulated in HIV-1–infected cells. The HSP90 inhibitor, geldanamycin causes suppression of TRAP1 (Kroemer and Blomgren, 2007).

Role of PINK1 in mitochondrial respiration and protein degradation

PINK1 (PTEN induced putative kinase1); PTEN is phospho & tensin homology. PINK1 gene located on chromosome # 1p35-36 and exists in form of dimer. A significant proportion of PINK1 has been localized to both the inner and outer mitochondrial membranes (Charleen, 2010;

Silvestri *et al.,* 2005). *PINK1* encodes a 581-amino-acid protein with a predicted N-terminal mitochondrial targeting sequence and a conserved serine/threonine kinase domain (Charleen, 2010). Stress signaling, which is induced by ROS (reactive oxygen species) and artificially by hydrogen peroxide causes the transcription of PINK1 gene (ROS produces as a result of oxidative reactions in the mitochondria, for energy production). Then this protein causes the phosphorylation of TRAP1, which is its substrate (TNF receptor-associated protein 1) inside the mitochondria. To promote the cell survival PINK1 causes the phosphorylation of the mitochondrial molecular chaperone TRAP1. This infers the normal activity of PINK1 being the protection against cell death induced by oxidative stress. In PC12 cells due to H_2O_2-induced oxidative stress, over expression of wild-type PINK1 causes a significant reduction in the release of cytochrome c. The inhibitory effect of PINK1 on cytochrome c release is totally abolished by the catalytically inactive D362A mutation and the triple KDD mutations. The deletion in PINK1 considerably increases oxidative-stress-induced release of cytochrome c from mitochondria to the cytosol in PC 12 cells, which suggest that kinase activity of PINK1 is critically involved in the regulation of mitochondrial apoptotic pathway. PINK1/ Parkin pathway has a regulatory role in the mitochondrial morphology Parkin is a cytosolic substrate of PINK1. PINK1-deficient cells are, unable to recruit Parkin followed by an impairment of mitochondrial functions (McBride *et al.,* 2008; Poole *et al.,* 2008)**.** The mitochondrial dysfunction caused by loosing PINK1 is evidently restored by Parkin, it is uncertain whether a direct molecular communication between PINK1 and Parkin is necessary for efficient removal of injured mitochondria. Pink1 & Parkin, both work in a common pathway to maintain the integrity of mitochondria & cell survival (Clark *et al.,* 2006; Yang *et al.,* 2006**).** The PD-linked PINK1 G309D, L347P mutations impair the PINK1 activity of promoting TRAP1 phosphory-lation and cell survival, but W437X mutations to some extent. Due to harsh mitochondrial injury, PINK1 due to its interaction**s** with Parkin facilitates aggregation and clearance of depolarized mitochondria**.** PINK1 protein is modified by, post-translational modification and localized into mitochondria. Excess expression of full-length PINK1 is compulsory for mitochondrial Parkin recruitment (Charleen, 2010). Full length PINK1 pre-protein (~63 kDa) is cleaved to a 'mature' form (54 kDa) by an unknown protease. PINK1 mRNA is expressed in human tissues, with highest expression in muscles. (Kaczmar *et al.,* 2008; Gandhi *et al.,* 2006; Muqit *et al.,* 2006). In PD mostly mutations of PINK1 are found within the ATP binding site of kinase domain. Mutations in N-terminus & C-terminus of protein are also important for optimal activity of kinase domain. Mutated PTEN (phospho and tensin homology) induced putative kinase 1 *(PINK1)* gene causes an autosomal recessive form of Parkinson disease (PD), a neurodegenerative disease of dopamenergic neurons of mid brain due to decrease in mRNA of PINK1 in them which leads to the decrease phosphorylation of TRAP1. PINK1 is one of the genes out of 7 mitochondrial genes encoding complex I subunits of ETC. In idiopathic PD abundance of mutations are present in a very narrow region of the mitochondrial complex I gene, ND5 substantia nigra of PD brains (Gegg *et al.,* 2009; Park *et al.,* 2006). PINK1 is an essential mitochondrial quality control regulator, which promotes the maintenance of

mitochondrial respiration. The mitochondrial electron transport chain (ETC) impairment and an increased deletion rate of mitochondrial DNA (mtDNA), which encompasses some of the ETC sub-units due to over production of ROS in case of genetic ablations of PINK1. PINK1 is involved with the respiratory complex 1 (NADH : ubiquinone oxidoreductase) of ETC so because of inhibition of complex1 by the neurotoxin, MPTP (1-methyl-4-phenyl tetra hydropyridine), produces parkinsonism in humans. Suggesting that one of the main causes of neuron loss and motor impairment in PD is toxin-induced mitochondrial stress in dopaminergic neurons but the actual mechanism by which PINK1 mutations lead to neurodegeneration is unknown (Charleen, 2010; Gegg *et al.*, 2009; Piccoli *et al.*, 2008).

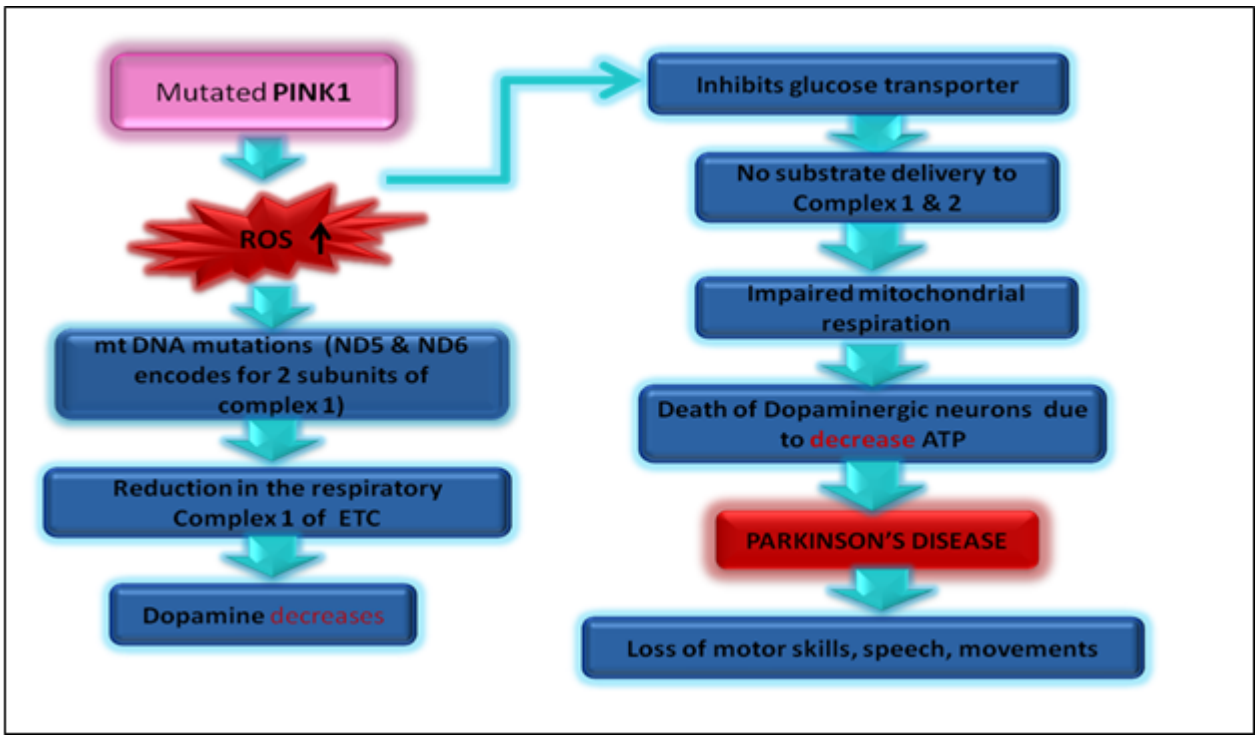

Figure 3.0: Impaired Mitochondrial Respiration due to mutated PINK1.

MPTP is transformed in to a derivative, 1-methyl-4-phenylpyridinium (MPP+) and this derivative inhibits complex I of the mitochondrial ETC. Epidemiologic studies also propose mitochondria-toxic pesticides may increase PD risk. Rotenone is also complex I inhibitor (Swerdlow, 2009). Pham *et al.*, 2000 states that an increased production of mitochondrial reactive oxygen intermediates (ROI) due to the impaired activity of respiratory chain ultimately proceeds with physiological processes namely apoptosis, being a part of both cases i.e., normally the aging process and as well during the abnormal mitochondrial pathogenesis. Flow cytometry procedures revealed that there is a marked relationship between the generation of mitochondrial ROI and disordered functions of respiratory chain complexes. The genetics of mitochondria also gave a helping hand to the investigators for the identification of several rare disorders as

likely arising from mutation of mitochondrial DNA (mtDNA). This indicates that deficiency of complex 1 in electron transport chain defects due to mutated PINK1 leads to degeneration of dopaminergic neurons in the central nervous system. In an experimental PINK1 compromised model from a human dopaminergic neuroblastoma cell line with shRNA leads to decreased mitochondria membrane potential as compared to wild-type. This also causes decreased mitochondrial respiration, because provision of substrates for mitochondrial complexes I and II partially declines and shortfalls. The lack of substrate supply also augmented the plenty of the oxidized forms of NADH and FAD^{2+} in mitochondria lacking functional PINK1 **(**Ray, 2009). The F(1)-ATP synthase become reverse When mitochondrial respiration decreases and start using ATP, helping to retain the mitochondrial membrane potential. The density of mitochondrial cristae is increased by IF (1) over expression and decreased by IF (1) repression; and IF (1) over expression increases the formation of ATP syntheses complexes and increases F (1) F(o)-ATP synthase activity. (Campanella *et al.,* 2008)**.** Several studies on the loss of Pink1 or parkin in Drosophila show significant mitochondrial swelling (Clark *et al.,* 2006; Park *et al.,* 2006). In a mice with germline deletions of the $PINK1^{-/-}$ gene leads to impairment of mitochondrial functions. At 3-4 and 24 months under quantitative electron microscopic study of $PINK1^{-/-}$ mice, shows that, there are no changes in the ultrastructure or the total number of mitochondria but number of larger mitochondria is selectively increased as well as impaired mitochondrial respiration only in the dopamenergic neurons but after two years later also in the cerebral cortex . This indicates that aging can aggravate mitochondrial dysfunction in these mice and mitochondrial respiration defects can be induced in the cerebral cortex of $PINK1^{-/-}$ mice by cellular stress (Gautier *et al.,* 2008). The phenotypes of PINK1 or Parkin mutant Drosophila are very similar, PINK1 is not capable to rescue the faults caused by the lack of parkin but the over expression of parkin rescues the mitochondrial pathology induced by the knocked out Pink1. Mammalian PINK1-lacking cells confirm fragmented and truncated mitochondria. The molecular interaction between Parkin and PINK1 is conserved in mammalian cells (Lutz *et al.,* 2009; Exner *et al.,* 2007). As mutant PINK1 leads to impaired proteasome function & increase in alpha-synuclein aggregation, which leads to the toxicity in neurons and mitochondrial dysfunction plays an important role in initiation of apoptotic (Budd *et al.,* 2000; Radke *et al.,* 2008).

Protein aggregation due to mitochondrial dysfunctioning plays an important role in the pathogenesis of PD. α-synuclein is a fibrillar aggregation of protein that is a main constituent of Lewy bodies and is supposed to contribute to PD due to their toxic effects. Although α-synuclein is mostly cytosolic protein but in numerous transgenic mouse models mitochondrial abnormalities were observed that either lacks or over express wild-type or mutant α-synuclein (Poon *et al.,* 2005; Martin *et al*,2006). These studies indicate that in the mitochondria, α-synuclein has a physiological role because α-synuclein interacts with mitochondrial proteins of dopamenergic neurons (Ellis *et al.,* 2005). In a Drosophila loss of PINK1 causes an increase of α-synuclein aggregation in the mitochondria but due to over expression of PINK1 α-synuclein, accumulation is suppressed (Castro *et al.,* 2010; Liu *et al.,* 2009). Moreover, in case of the oxidative mitochondrial stress and to keep away the toxic aggregation of α-synuclein via oxidation, the chaperone DJ-1 has been admired to undergo relocation (Hayashi *et al.,*

2009; Junn *et al.,* 2009; Shendelman *et al.,* 2004). Omi/HtrA2 and DJ-1 along with PINK1 offer protective functions within mitochondria to maintain the integrity of neuronal cells (Plun *et al.,* 2007; Strauss *et al.,* 2005). PINK1, Parkin, and DJ-1 formed a complex known as *PPD* complex, which promotes the degradation of unfolded mitochondrial proteins (Xiong *et al.,* 2009). It has been evidently suggested that PINK1 deficiency results in diminished long term viability in human neurons, whose death relies in the apoptosis pathway of mitochondria. (Kaczmar *et al.,* 2008; Unoki *et al.,* 2001). So PINK1 also helps in the mitochondrial respiration and ATP production as well as in protein degradation.

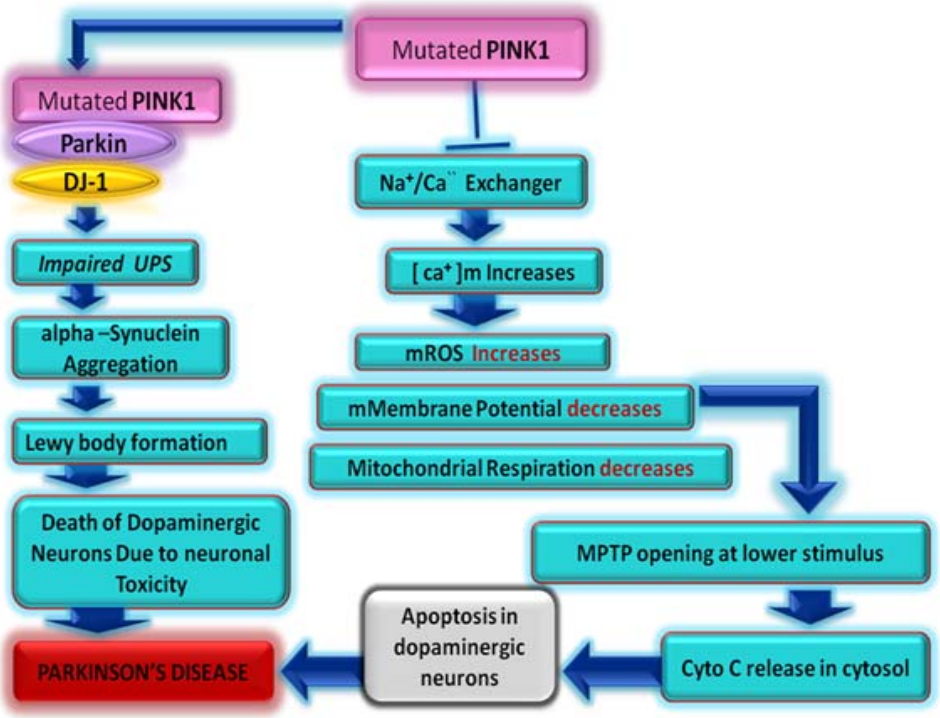

Figure 3.1: Mutated PINK1 is associated with the pathogenesis of Parkinson's *Disease*

Contribution of PINK1 in Calcium Signaling

PINK1 plays a significant role in the Calcium Signaling. Mitochondrial Ca^{2+} accumulation is a tightly controlled process. Deregulation of Mitochondrial Ca^{2+} induces death of neurons. PINK1 physiologically regulates the calcium efflux from the mitochondria through the ATP dependent mitochondrial Na^{+}/Ca^{2+} exchanger. PINK1 deficiency causes to malfunction of a Na^{+}/Ca^{2+} exchanger due to lack of ATP, thus avoiding the proper calcium efflux from the mitochondria induces mitochondrial accumulation of free calcium and mitochondrial calcium overload. (Abramov *et al.,* 2007; Ray, 2007; Gandhi *et al.,* 2009). Calcium overload causes the stimulation of reactive oxygen species (ROS) production. Then ROS inhibits the glucose transporter which causes the decrease delivery of substrate to the Complex 1 of the ETC. This leads to impaired respiration in the

mitochondria. Increased ROS production also causes decreased mitochondrial membrane potential and ATP production. This reduced mitochondrial membrane potential induces the MPTP opening at lower stimulus due to decreased threshold require by the cells to open MPTP. Cytochrome c releases through the MPTP into the cytosol causes apoptosis of the cell. Mitochondrial membrane potential ($\Delta\psi_m$) is a pointer of mitochondrial state. Mitochondrial Ca^{2+} in neuronal cells death highlighting the role of Ca^{2+} in the pathogenesis of Parkinson's diseases (Szabadkai *et al.,* 2006 ; Gandhi *et al.,* 2009; Ray, 2009). A neuronal cell model of PD analyzed for cell survival, mitochondrial activity and calcium flux. These cells infected with virally-delivered Pink1. Under confocal and electron microscopy mutant (W437X) shows alterations in mitochondrial function but not the wildtype Pink1. This effect is linked with increased intracellular calcium levels. In a Parkinson's disease model of neuron cell a dysfunction is induced in mitochondria via mutant PINK1 (Mattson, 2007; Gandhi, 2009). In the PINK1 KD neuroblastoma cells the significantly reduced oxygen consumption is observed because lack of substrates for complex I in these cells, inhibits respiration. (Gandhi *et al.,* 2009; Szabadkai *et al.,* 2006). As a decreased gulucose uptake in the neurons of human and mouse is evidently associated with deficient PINK1. In these cells it is also observed that the rate of basal mitochondrial ROS (mROS) production was also significantly higher in PINK1 KD cells (Scheele *et al.,* 2007; Anantharam *et al.,* 2007). In another experiment, the PINK1 KD neurons has high mitochondrial Ca^{2+} level followed by a stepwise mitochondrial depolarization. Which is associated with lowering of $\Delta\psi_m$. This mitochondrial depolarization was prohibited by the administration of cyclosporin A (CsA). Cyclosporin A basically inhibits mPTP but pyruvate and methyl succinate which are the substrates of respiratory chain are not able to avoid the mitochondrial depolarization induced by calcium in PINK1 KD cells (Mattson, 2007).This indicates that reduced substrate delivery causes the impairment of respiration and reduced $\Delta\psi m$ in cells that lack PINK1 (Scheele *et al.,* 2007 ; Ray, 2009, b; Gandhi *et al.,* 2009). The PINK1 also interacts with the calcium sensing molecule NCS-1. This regulates the neurotransmitter release. Thus mitochondrial calcium functions as a neuronal pro-survival factor (Nakamura *et al.,* 2006). PINK1 also has an additional indirect role in calcium signalling in zebrafish (Petko *et al.,* 2009).

Parkin / E3 ligase and PINK1'S Relation

Parkin plays a very important neuroprotective role along with Pink1 as well as with DJ-1 & alpha synuclein. Parkin is the gene product of PARK2 & it is also called E3 ligase of ubiquitin proteasome pathway. Which is involved in the degradation of neurotoxic proteins (Vercammen *et al.,* 2006). Chung and colleagues state that the direct phosphorylation of Parkin by PINk1 on Thr175, the kinase domain of which faces the cytosol is responsible for the recruitment of Parkin. Parkin has an N-terminal ubiquitin like domain & it is a component of multiple proteins in of ubiquitin proteasome system (UPS) (Ayako *et al.,* 2005). Most of the neurodegenerative diseases are due to degeneration of specific neuronal population. Due to misfolding, aggregation & then their neurotoxic effects on neurons. (Yang *et al.,* 2006). Mutations of PINK1, Parkin & DJ-1 leads to autosomal recessive form of Parkinson's disease (PD). (Xiong *et al.,* 2009). As mutations of these genes cause accumulation of Parkin's substrate proteins which leads to toxicity in the neuro (Vercammen *et al.,* 2006). Parkin has both degradative & nondegradative roles. As an

E3 ubiquitin ligase, Parkin causes polyubiquitnation of numerous proteins (misfold /denatured, short lived or regulatory) for their degradation by ubiquitin proteasome system (UPS). A signal ubiquitin molecule attached with these proteins before their degradation by UPS. Many studies suggest that Pink1 and Parkin, both work in a common pathway to maintain the integrity of mitochondria and cell survival. (Clark *et al.,* 2006; Yang *et al.,* 2006). Park *et al.,* 2006 stated that mutants of pink1 and Parkin share marked phenotypic similarities. Mitochondrial dysfunctioning also contributes in the etiology of Parkinson's disease. In Hela cells the down regulation of Pink1 due to RNA interference causes many abnormalities in the mitochondria of the cell like altered mitochondrial membrane potential and abnormal mitochondria's morphology which leads to cellular stress & pathological phenotype (Exner *et al.,* 2007). Similarly drosophila in which Pink1 depletion leads to dysfunctioning of mitochondria, energy depletion, degradation of muscles & dopaminergic neurons of substantia nigra is rescued by over expression of Parkin. Here this thing is important that Pink1 is unable to rescue or overcome the abnormalities in the drosophila, in which Parkin is knocked out. (Clark *et al.,* 2006 ; Park *et al.,* 2006). Parkin suppresses the death of dopaminergic neurons induced by oxidative stress as well as due to unfolded protein stress. (Yang *et al.,* 2006; Lo Bianco *et al.,* 2004). Pink1 along with Parkin & DJ-1 form a complex known as PPD complex. This promotes polyubiquitnation & then degradation of Parkin's substrates. (Xiong *et al.,* 2009). Parkin-associated endothelin-like receptor (Pact-R), alpha synuclein & alpha synuclein binding protein (synphilin1 proteins) are substrate of Parkin. (Yang *et al.,* 2003; Lo Bianco *et al.,* 2004). These substrate proteins get accumulated in case of genetic ablations of Pink1 because Parkin mediated degradation of misfolded proteins is enhanced by Pink1. Genetic ablations in the gene of alpha synuclein are linked to the early onset of familial form of Parkinson's disease. As Parkinson's disease associated alpha synuclein mutant protein aggregations are major component of cytoplasmic inclusions known as Lewy's bodies in dopaminergic neurons of substantia nigra. (Xiong *et al.,* 2009). Along with Pink1 protein Parkin acts as a central player in the pathogenesis of Parkinson's disease as it suppresses the neurotoxicity due to accumulation of Putative G protein coupled transmembrane receptor (Pact-R) and alpha synuclein. Parkin degrades proteins with aberrant conformation and is associated with autosomal recessive Juverile Parkinsonism (AR-JP) (Yang *et al.,* 2003; Rankin *et al.,* 2005). In PARK2 gene recessively inherited duplications / deletions & point mutations are the most common cause of early onset of Parkinsonism (Myhre *et al.,* 2008). Rankin *et al.,* 2005 cloned & expressed human Parkin in E-coli and examined Parkin mediated ubiquitnation in an ubiquitnation invitro assay. They observed that E3 ligase activity is an intrinsic function of Parkin protein and does not require post translation modifications or associaton with cellular protein other than an E3 ligase.

Activation of NF-KB signaling by PINK1 & Parkin

One pathway of activation of NF-KB through IKK complex is called "classical," or canonical, pathway. Other pathway of NF-KB is called alternative," or noncanonical, pathway (Zarnegar *et al.,* 2007).

NF-KB is a key player to maintain the integrity of neurons. As NF-KB is present throughout the nervous system. NF-KB is a transcription factor which regulates many physiological functions and also involved in the pathogenesis of disease. PINK1 activates and enhances the E3 ligase activity of Parkin by phosphorylating it directly **(**Sha *et al.,* 2009**).** This further causes the ubiquitnation of NEMO and TRAF2 (Chen, 2005). Activation of NF-KB in neurons promotes their survival and activation of NF-KB in glial and immune mediated cells leads to pathological inflammation. Physiologically Parkin causes the activation of signaling cascade for neuroprotection. This neuroprotection is abolished when genetic ablations of pink1 or Parkin occurs because these mutant genes are unable to activate NF-KB and leads to the pathogenesis of Parkinson's disease, Epilepsy and AD **(**Sha *et al.,* 2009).Activation of NF-KB causes the transcription of those genes which supports pro-survival activities in neurons. These are anti apoptotic proteins such as Bcl-2. This shows that Parkin has cytoprotective role.

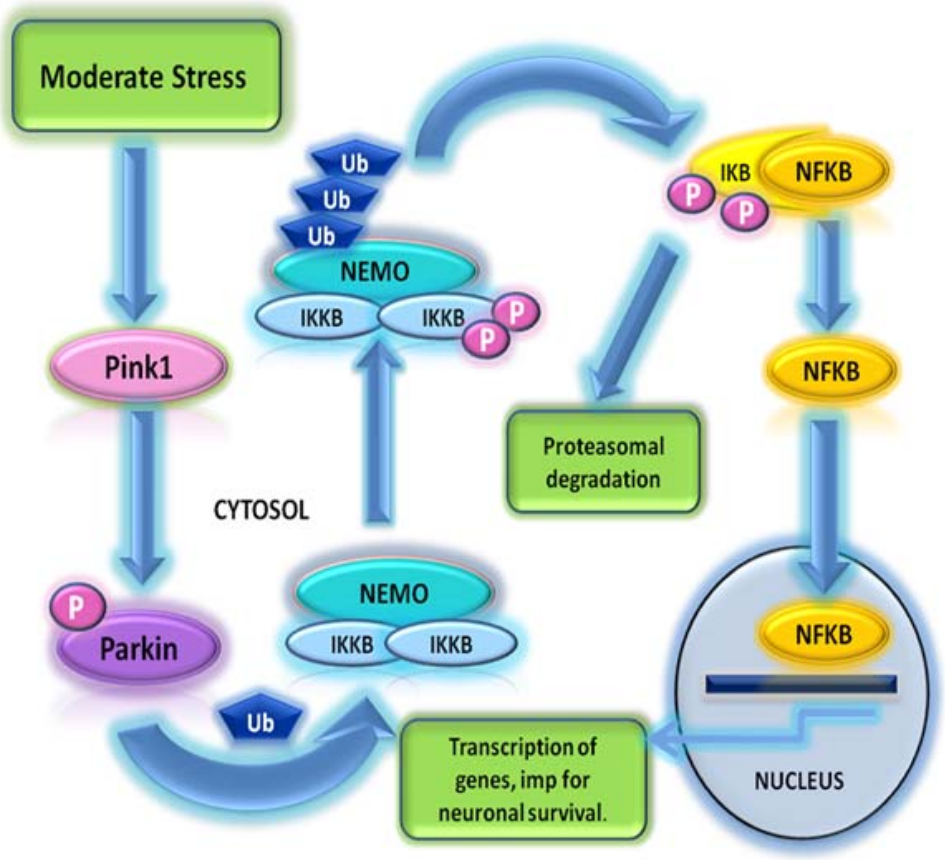

Figure.4.0: Activation of NFKB signaling by PINK1

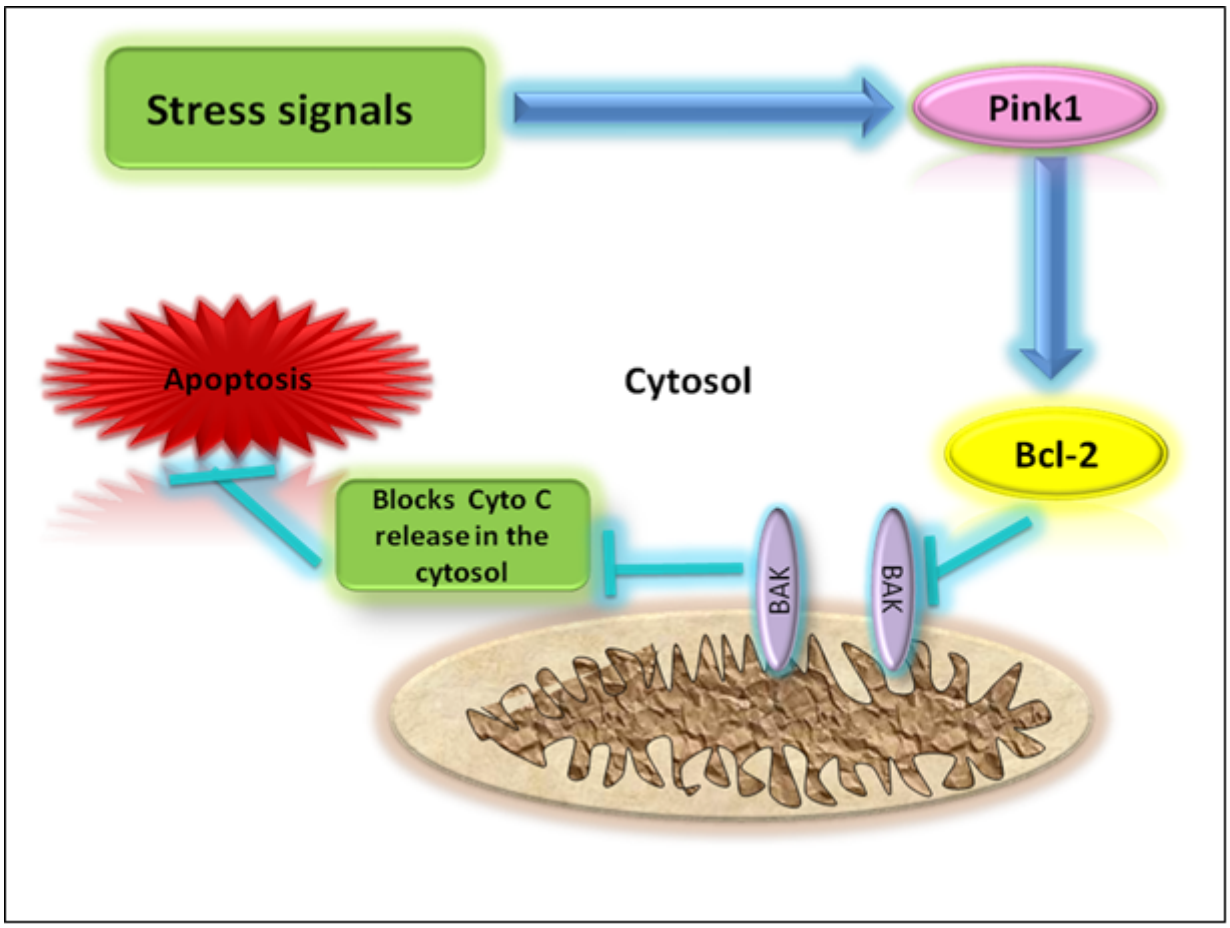

Figure.4.1: Neuronal survival by activation of antiapoptotic Bcl-2 through PINK1

Recent studies indicated that Parkin has cytoprotective function in cell cultures and in animal models by activation of NF-KB because mutated Parkin in HEK293T cells is unable to trigger NF-KB pathway **(**Henn *et al.,* 2007**).** NF-KB dependent genes transcription is stimulated by Parkin in HEK293T cells. These genes further sustain the neuronal survival. In SH-SY5Y cells, activation of NF-KB pathway is essential for protection of neurons from apoptosis. It is observed that in HEK293T cells Parkin forms a complex with IKK and TRAF 2. Analysis show that Parkin causes activation of NF-KB pathway which is essential for the neuronal survival.IKK complex consists of two subunits. IKK regulatory subunit also called NEMO (NF-B essential modifier) and a catalytic subunit IKKB **(**Sha *et al.,* 2009**).**The NF-B pathway is a paradigm for ubiquitylation mediated dependent degradation**.** Ubiquitnation in the NF-KB pathway includes targeting of IB for degradation by binding of Ubiquitin moiety (Chen, 2005; Krappmann and Scheidereit, 2005). Naoi *et al.,* 2009 stated that they evaluate the cytoprotective activity of Parkin by two stressors relevant to, rotenone induces inhibition of complex I of the electron transport chain and glutamate induces excitotoxicity. Both stressors drastically increased the quantity of Parkin specific mRNA in cultured neuroblastoma cells as well as primary neurons, consequential in an increased expression of Parkin protein. Pathogenic muated parkins do not exhibit a complete loss of function when over

expressed in cultured cells. However a blockage of the NF-B pathway leads to a loss of protective activity of parkin in dopaminergic neurons, this proceeds via the modulation of NF-KB pathway; Parkin initiates a neuroprotective plan under lowered-level and moderate stress. The promoter region of parkin associates numerous stress response elements. The experimental model predicts that the surplus expression of parkin remains sufficient to activate NF-B cascade and the parkin's phosphorylation status is very important for its activity (Yamamoto *et al*., 2005). Under the moderate stress activated PINK1 causes the phosphorylation of Parkin in the cytosol of dopaminergic neurons and then activated Parkin causes the lys-63 linked ubiquitnation of NEMO and TRAF2 in the form of polyubiquitin chain by adding Ubiquitin moiety **(**Sha *et al.,* 2009**).** These polyubiquitin chains contain lys 63 / Gly 76 isopeptide linkages. This isopeptide linkage is between the glysine 76 amino acid at the C-terminal of Ubiquitin moiety and Lysine 63 amino acid of substrate protein (Moore, 2006). This further leads to the phosphorylation and activation of catalytic subunit of IKK complex that is IKKB. This activated subunit causes inhibition of IKB by its phosphorylation and Lys48-linked polyubiquitylation. In its inactivated version NF-KB binds with IKB but when IKB is inhibited by IKK complex NF-KB gets free. The polyubiquitylated IKB leads to proteasomal degradation and activated NF-KB translocates in the nucleus for the transcription of antiapoptotic genes for survival of dopaminergic neurons.

PINK1, Parkin and JNK pathway: Two's a company, Three's a crowd

JNK pathway is also controlled by PINK1 and Parkin in dopaminergic neurons. Under oxidative stress PINK1 causes phosphorylation of Parkin. This activated Parkin causes the activation of Akt by its phosphorylation. This activated Akt blocks the JNK pathway. NF-kappaB promotes survival, whereas JNK enhances stress induced apoptosis in dopaminergic neurons of substantia nigra and leads to pathogenesis of Parkinson's disease (Chang *et al.,* 2006). Administration of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in SH-SY5Y human neuroblastoma cells *in vitro* and in mice *in vivo causes* activation of JNK pathway by phosphorylation JNK. This causes the activation of caspases which leads to apoptosis of these cells. The dopaminergic neurons in the substantia nigra of brain are selectively lost due to the apoptosis induced by the neurotoxin MPTP. Treatment of mice with SP600125 an inhibitor of JNK pathway lead to a reduction in phosphorylation of JNK. This decreased the apoptosis of dopaminergic neurons and restored the dopamine level in the MPTP induced Parkinson's disease (Wang *et al.,* 2004; Xia *et al.,* 2001). This indicates that inhibitor of JNK pathway protects dopaminergic neurons from apoptosis induced by neurotoxin (Peng *et al.,* 2004). Mutant Parkin in the Drosophila leads to decreased levels of dopamine in their brain because of highly activated JNK pathway in Parkin mutant flies. These results suggest that Parkin inhibits the JNK signaling pathway and loss of cytoprotective functions of Parkin, in Parkin mutant flies, there is an up regulation of JNK pathway. This further leads to autosomal recessive juvenile Parkinsonism (Cha *et al.,* 2005). Cha *et al.,* 2005 also stated that Parkin inhibits JNK pathway in the Drosophila and human neuroblastoma cells. This indicates that Parkin down-regulates the proapoptotic JNK pathway.

Role of PINK1 in PI3k/Akt pathway

As a result of oxidative stress PINK1 causes activation of Parkin. This activated Parkin causes phosphorylation of Akt which causes activations of many other molecules by

phosphorylating them such as GSK3, FoxoA2 to hinder the apoptosis (Rise *et al.,* 2006). PI3K/Akt are also important targets of Parkin. Activation of PI3K/Akt pathway protects the dopaminergic neurons of substantia nigra from the apoptosis (Yuan J. and Yankner, 2000). Activated parkin blocks the endocytosis of neurotropin receptor which is present on the cell membrane. Neurotropin receptor further causes the activation of PI3K. This PI3K acts on its substrate, phosphatidylinositol-4,5-bisphosphate. This pathway leads to the phosphorylation plasma membrane phosphatidylinositol-4,5-bisphosphate in to phosphatidylinositol- 3,4,5-P3 by PI3K (Vanhaesebroeck, 2001). This conversion plays a very important neuroprotective role because it triggers the activations of Akt. Kittappa *et al.,* 2007 stated that Akt protects dopaminergic neurons of a mouse from neurotoxin 6-hydroxy-dopamine induced apoptosis. As it acts as antioxidative stress factor (Mei *et al* 2009). In another experiment it is observed that amount of activated Akt decreases in a Parkin mutant Drosophila and concludes that Parkin plays a very important role in the phosphorylation of Akt in neuronal cells (Rise *et al.,* 2006). Experiments with pharmacological inhibitors of Akt also demonstrated that Akt mediates PI3-K-dependent survival of neurons (Dudek et at, 2007). FoxoA2 is one of the substrate of Akt and an important transcription factor. Loss of FoxoA2 also causes selective loss of neurons. In a mice having only one copy of the gene for FoxoA2 leads to decrease neuronal survival (Kittappa *et al.,* 2007). These evidence shows that PI3K/Akt pathway plays a very important role in protection and survival of dopaminergic neurons against oxidative stress induced apoptosis with the help of activated PINK1 and Parkin. Phosphorylated Akt also blocks the polymerized Bax, present on the mitochondrial membrane to prevent the release of cytochrome c, smac in to cytosol from mitochondria.

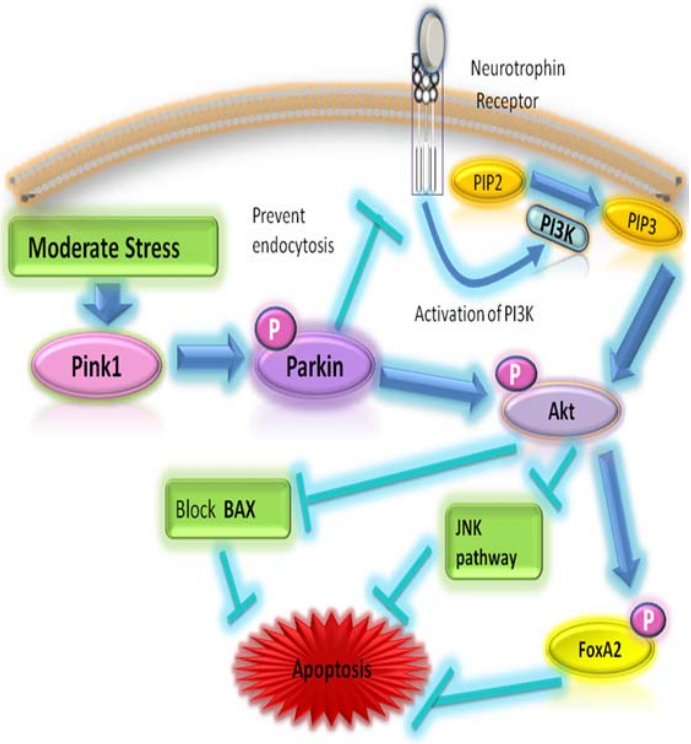

Figure 5: Activation of PI3k / Akt signaling.

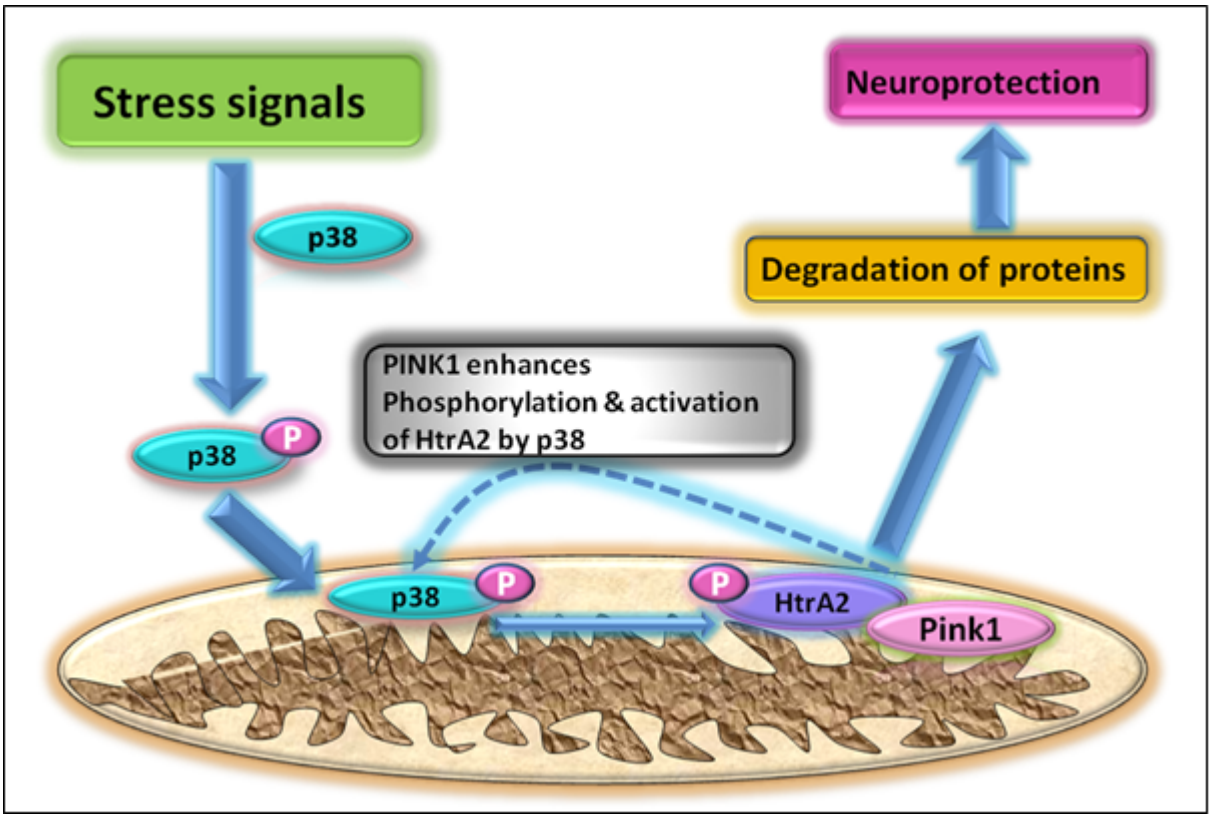

Figure 6: Ubiquitin independent degradation of mitochondrial proteins by HtrA2.

Mitochondrial Protease/HtrA2 and PINK1's Job

High temperature requirement A2 (HtrA2) also called Omi, is one member of HtrA family. HtrA2 is a serine-25 protease HtrA2 of 485 amino acids. In humans HtrA2 is localized to inner mitochondrial space (IMS). As it has mitochondrial targeting signal. Mammalian has cell proptective and pro-apoptotic role (Alnemri, 2007; Koonin and Aravind, 2002). Serine protease in the mitochondria which ensures the mitochondrial protein quality is ubiquitin independent. Activity of HtrA2 causes removal of accumulated and misfolded protein from inner mitochondrial space because of inhibition of UPS (Radke *et al.*, 2008). PINK1 has been identified as an important regulator for the activity of mitochondrial associated stress-protective serine protease, HtrA2 (Alnemri, 2007). P38 dependent phosphorylation of HtrA2/Omi at serine 142 is mediated by PINK1. This phosphorylation of HtrA2/Omi being induced by stress boosts-up its activity. Finally providing a significant protection against Parkinsonism (Plun-Favreau *et al.*, 2007). The classification of HtrA2/Omi as PARK 13 due to presence of two mutated alleles of patients suffering from Parkison's disease (Alnemri, 2007). PINK1 dependent phosphorylation of HtrA2 regulates the proteolytic activity of HtrA2 and makes the cell resistant to mitochondrial associated stress (Plun-Favreau *et al.*, 2007). HtrA2 has no critical role in the PINK1 and Parkin

interactions (Tain *et al.,* 2009). PINK1 and HtrA2 protect the mitochondria from stress within the cell to maintain the integrity of the cell. Without controlling the stress, cell can't function properly. Genetic ablations of PINK1 and HtrA2 are very important aspect in the development of Parkinsonism neuro-degenration (Martins *et al.,* 2004). In patients suffering from Parkinson's disease, mutations of PINK1 C573R and Y431H show decrease ability of PINK1 to phosphorylate HtrA2 in the postmortem brain tissue as compared with normal neurological control (Plun-Favreau *et al.,* 2007). The protease activity of HtrA2 protects the mitochondria from stress by reducing mitochondrial ROS (Krick *et al.,* 2008). Mutated HtrA2 is unable to perform its protease activity while mutated, this leads to the accumulation of mitochondrial unfolded proteins, cause defects in mitochondrial respiration and increases the level of ROS ; resulting in the ultimate neuronal death (Moisoi *et al.,* 2009). Mutated HtrA2 as well show some phenotypic similarities with PINK1. Suggesting that both of them help in maintaining the mitochondrial integrity and both of them are part of stress sensing pathway (Alnemri, 2007). In SH-SY5Y human neuroblastoma cells PINK1 forms complex with HtrA2 but not in HtrA2 deficient cells. This shows that HtrA2 form complex with PINK1 and both are part of stress sensing pathway (Plun-Favreau *et al.,* 2007). Emad, 2007 reported that HtrA2 acts downstream to PINK1 but in a pathway parallel to Parkin. Mutated HtrA2 protease domain in mice shows motor neuron degeneration (mnd2) leads to its muscles wasting and neuro-degenration. In another experimental HtrA2 knocked out mouse shows progressive neurodegenration of dopamine neurons and exhibit phenotype of Parkinsonism, abnormal mitochondria and reduce life span as compared to normal mice. This highlights the role of HtrA2 to maintain the mitochondrial function and integrity of neuronal cells rather than its pro-apoptotic function in the neurons (Liu *et al.,* 2007). Like PINK1 mutant flies, HtrA2 mutant flies also show reduce flight and climbing abilities and this is completely restored in HtrA2 rescued flies. phenotypicaly over expression of PINK1 is suppressed by loss HtrA2 of but over expression of HtrA2 is not suppressed by PINK1 mutations (Alnemri, 2007). Loss of HtrA2 leads to only mitochondrial alterations in aged flies (Tain *et al.,* 2009). Proteolytic HtrA2 has a conserved IAP binding domain, homologous to mammalian smac/DIABLO (Koonin and Aravind, 2002). Due to pro-apoptotic stimulus such as UV radiations HtrA2 releases from mitochondria into cytosol causes the active cleavage of IAP (Yang *et al.,* 2007). IAP (Inhibitor of apoptosis proteins) which further causes the activation of caspases leads to apoptosis (Cilenti *et al.,* 2004).

PINK1, Parkin's interaction with DJ-1

Biochemical function of DJ-1 rema**in**s unidentified **(**Goldberg_*et al.,* 2005**)**. Genetic ablations of DJ-1 also contribute in the pathogenesis of Parkinsonism, so it is also called PARK 7. *DJ-1* is important for proper mitochondrial function and acts downstream of, or in parallel to, *pink1* and maintains the mitochondrial integrity (Hao *et al.,* 2010). Mutations in the *DJ-1* gene are source of early-onset familial Parkinson's disease (Bonifati *et al.,* 2003; Dodson and Guo, 2007**).** In an animal model reduced function of DJ-1A due to RNA interference leads to decrease phosphorylation of Akt. This shows that PI3K/Akt signaling becomes impaired due to down-regulation of DJ-1A. Similarly in mammals when PI3K/Akt signaling becomes impaired due to mutated DJ-1. This leads to DJ-1-associated disease pathogenesis (Yang *et al.,* 2005). Parkin, PINK1, and DJ-1 formed a complex known as *PPD* complex. In an experiment this complex promotes ubiquitination of Parkin substrates like alpha-Synphilin-1 in neuroblastoma cells and

human brain lysates which resulted in their degradation. Genetic ablation of any of these genes, Pink1 or Dj-1 leads to reduced ubiquitination of Parkin substrates as well as decreased degradation and increased accumulation of abnormally expressed Parkin substrates (Xiong *et al.,* 2009). Mutant DJ-1 mice show dopamine reuptake dysfunction and have increased sensitivity to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) (Hao *et al.,* 2010). In transfected cells and in Drosophila DJ-1 protects both cells from oxidative stress (Bonifati *et al*., 2003; Meulener *et al.,* 2005). Silencing of *Dj-1* in mouse and PINK1 in Drosophila causes mitochondrial dysfunction and increased sensitivity to oxidative stress (Goldberg *et al.,* 2005**).** Mutually, Parkin, PINK1, and DJ-1 protect cells against oxidative stress by way of a common mechanism, portentous potential functional relations between the three proteins. These results propose that the Parkin/PINK1/DJ-1 (PPD) complex plays an important role in degradation of denatured and regulatory proteins through UPS (Xiong *et al.,* 2009). In SH-SY5Y neuroblastoma cells localization of the PPD complex is determined by immunostaining and cellular fractionation. By fractionation analysis of SH-SY5Y cells these proteins Parkin, PINK1, and DJ-1 are detected in both the mitochondrial and the cytosolic fractions and colocalization of Parkin, PINK1, and DJ-1 is largely observed in the cytoplasm of cultured primary human neurons.PPD complex is more abundant in the cytosolic fraction as compared to mitochondrial fraction and only a little amount of the PPD complex is present in the mitochondrial fraction (Xiong *et al.,* 2009). PPD promotes degradation of Synphilin-1 by means of the ubiquitin-proteasome system. WT DJ-1(DJ-1WT) and pathogenic loss of function mutant DJ-1L166P are utilized in the experiment to define the promising role of DJ-1 in the PPD complex. The presence of DJ-1WT upregulates the ubiquitination of Parkin not disregarding the fact that steady-going level of Parkin is not altered. Tied in findings were prevailed with Synphilin-1. These findings proposed that DJ-1WT, and not PD-associated DJ-1A39S, is involved in stabilizing the level of PINK1. DJ-1 potentially modulates PINK1 in the PPD complex because the cells expressing DJ-1 result in the systematically increased balance level of PINK1.One of the possible role of DJ-1 in PPD complex is to stabilize PINK1, and DJ-1 also assists however it is not vital for the action of E3 ligase complex. Parkin, PINK1 and DJ-1 all these three proteins therefore as well solely work out with the protection against oxidative stress (Menzies *et al.,* 2005; Moore *et al.,* 2005).

PD and signaling cascades: current insights

TGF signaling

It is interesting to note that transforming growth factor-beta (TGF-beta) is significant for maintenance of structural integrity of dopaminergic neurons. TGF-beta2 haploinsufficiency results in defective neurons Andrews *et al*, 2006; Vawter *et al*, 1996; Roussa *et al*, 2009. It has recently been explored that neurotrophic factor and transforming growth factor β1 are involved in the neuroprotection Gonzalez-Aparicio *et al*, 2010. In agreement with the same assumption it was also noted that CD4+CD25+ regulatory T cells triggered the up regulation of neurotrophic factor and transforming growth factor β1 Reynolds *et al*, 2007.

Wnt signaling

Compelling evidence indicates that Dishevelled (DVL) interacts with and triggers the activation of small GTPases structurally similar to the LRRK2 Roc domain. It is obvious that LRRK2 Roc-COR domain and the DVL1 DEP domain are necessary for LRRK2-DVL1 interaction. Furthermore, co-expression of DVL1 escalated LRRK2 steady-

state protein levels. Outstandingly, LRRK2-DVL1-3 associations were impaired by the familial PARK8 mutation Y1699C. This is indicative of the fact that mutations underpin lack of association between DVL1 and LRRK2 and drive neurodegeneration Sancho *et al*, 2009. Another interesting piece of evidence is that cells transfected with Wnt5a (VMN-Wnt5a) generated 10-fold more dopaminergic neurons than did conventional FGF2-treated neurons Parish *et al*, 2008. On a similar note inhibition of negative regulators of Wnt signaling, glycogen synthetase kinase (GSK) resulted in an increment in the dopaminergic neurons Castelo-Branco *et al*, 2004

SHH signaling

It is important to note that Wnt1-lmx1a forms a novel autoregulatory loop and henceforth regulates dopaminergic differentiation concomitantly with the SHH-FoxA2 pathway Chung *et al*, 2009. It has been documented that expression of the transcription factor Gli1 within neurons is neuroprotective for dopaminergic neurons in vivo Suwelack *et al*, 2004. Contrary to this another documentation suggests lack of any association between mutations in SHH and pathogenesis of PD Bak *et al*, 2004. Another important crosstalk between SHH and FGF8 was dismantled and it was suggested that overexpression of nuclear receptor Nurr1 re-sensitized the cells to extrinsic signals of both of these ligands Kim *et al*, 2003.

PD and ATM

DNA damage results in activation of ATM that induces cell loss and apoptosis. On the contrary, suppression of ATM attenuates the ATM mediated cell loss Camins *et al*, 2010. It has been found that ATM gene product is required for cell survival and genomic stability after exposure to low labile iron concentrations. Keeping in view the safeguarding activities of ATM it is obvious that pharmacological manipulation of ATM activity might offer exciting avenues for rational drug design Edwin *et al*, 2005.

PD and PDGF

Platelet-derived growth factor BB (PDGF-BB) and Platelet-derived growth factor CC (PDGF-CC) are members of the PDGF family and are involved in the structural integrity and differentiation of dopaminergic neurons Tang *et al*, 2010; Mohapel *et al*, 2005.

miRNA and PD: therapeutic implications

It has lately been found that increasing the level of let-7 or miR-184 hampered pathogenic leucine-rich repeat kinase 2 (LRRK2) Gehrke *et al*, 2010. Another interesting piece of evidence is that reduction of the affinity of miR-433 to the 3' UTR up regulated FGF20 expression and enhanced expression of alpha-synuclein, which could in turn promote dopaminergic neurons degeneration. Nonetheless, the research group was unable to document association between rs12720208 and PD, or an effect of miR-433 variants on this disease de Mena *et al*, 2010.

Conclusion

It is getting increasingly essential to unravel negative regulators which relentlessly challenge integrity of neuron. A detailed mechanistic insight is unavoidable get a step closer to individualized medicine.

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