7

Cyclin-Dependent Kinase 5 – An Emerging Player in Parkinson's Disease Pathophysiology

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1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder that affects 1% of the general population at age 65, with the prevalence rising to 4-5% by age 85 (de Rijk *et al.* 1997). Symptoms of PD include muscle rigidity, resting tremor, bradykinesia, postural instability, speech impediment and cognitive decline. At the cellular level, PD is characterized by selective degeneration of dopaminergic neurons in the substantia nigra, and the presence of cytoplasmic inclusions known as Lewy bodies in the brains of PD patients (Levy *et al.* 2009). Despite the prevalence of PD, therapeutic agents that slow or halt disease progression are lacking. Current treatments for PD are highly limited and focus predominantly on symptomatic relief. For example, the most effective treatment for ameliorating PD symptoms is administration of levodopa, a precursor that is converted to dopamine in the brain, to restore dopamine levels in PD patients. Nonetheless, the efficacy of levodopa wears off with prolonged treatment, in addition to triggering dyskinesia as a side effect (Obeso *et al.* 2010, Poewe *et al.* 2010). There is thus an urgent need to elucidate the cellular mechanism underlying PD pathology for the development of more effective treatments.

The etiopathology of PD has remained largely enigmatic. Majority of the PD cases are idiopathic, although familial cases of PD with identifiable mutations also account for about 5% of all PD cases (Dauer and Przedborski 2003). Prior to the identification of these missense mutations, nonetheless, scientists have predominantly focused on the two known cellular hallmarks of PD, namely the degeneration of dopaminergic neurons and the presence of Lewy bodies. Since majority of the motor deficits exhibited by PD patients is reversed by elevating dopamine levels in the brain, it is believed that the motor symptoms of PD are due mostly to the loss of dopaminergic neurons in the substantia nigra (Obeso et al. 2010). This has sparked extensive research aimed at elucidating the mechanisms implicated in the degeneration of these neurons. Abnormality in various cellular processes have been linked to neuronal loss in PD, such as oxidative stress, mitochondrial dysfunction, aberrant proteasomal degradation and deregulation of the autophagy pathway (Levy et al. 2009). On the other hand, the precise role of Lewy bodies has become a little controversial. While protein aggregates and the presence of cytoplasmic inclusions have long been considered as toxic, recent evidence suggests that the aggregates may also exhibit neuroprotective roles by serving as traps for the toxic oligomeric forms (Rubinsztein 2006).

With the dawn of the molecular era, knowledge on the pathogenic mechanisms also shifted from detection of aberrant cellular processes to the identification of molecules implicated in the pathological pathways. Importantly, unraveling of mutations associated with familial cases of PD enabled the mapping of genes and signaling pathways to the dysfunction of various cellular processes. A number of genes were found to be mutated in familial PD, including α-synuclein, Parkin, PINK1, DJ-1, leucine rich repeated kinase 2 (LRRK2), ubiquitin-C-terminal hydrolase-L1 (UCH-L1), synphilin-1 and HtrA2/Omi (Schulz 2008). These studies also led to the identification of α-synuclein as the main constituent of Lewy bodies (Levy et al. 2009). This new wave of information prompted scientists to address the signaling events that are upstream of the elimination of the diseased neurons. Among the long list of molecules that were found to contribute to PD pathology, cyclin-dependent kinase 5 (Cdk5) has emerged as an important player through its implication in multiple cellular events that are altered in PD.

Cdk5 is a serine/threonine kinase that is essential for the migration, survival and differentiation of developing neurons (Cheung and Ip 2007, Dhavan and Tsai 2001). Activated through binding to its activator p35 or p39, Cdk5/p35 activity is important for the regulation of neuronal survival during physiological and pathological states (Cheung and Ip 2004). Cdk5 was first implicated in dopaminergic neuron loss in PD when injection of MPTP, a neurotoxin that selectively eliminates neurons in the substantia nigra, was demonstrated to increase Cdk5 activity (Smith et al. 2003). Inhibition of Cdk5 activity markedly attenuates MPTP-induced degeneration of dopaminergic neurons, thus revealing the involvement of Cdk5 in PD pathogenesis (Smith et al. 2003). Interestingly, Cdk5 expression is also associated with Lewy bodies (Takahashi et al. 2000). Since then, accumulating studies have identified additional Cdk5 substrates that may contribute to PD pathology. These findings revealed that Cdk5 regulates neuronal death through modulating multiple signaling pathways and cellular events in PD, and suggests that Cdk5 may emerge as a suitable target for development of PD therapeutics.

2. Cdk5 – a multi-faceted kinase

Cdk5 was identified based on sequence homology to cell cycle regulator cdc2 as a cdc2-related kinase (Lew *et al.* 1992, Meyerson *et al.* 1992) and also as a tau kinase (Kobayashi *et al.* 1993). Cdk5 is not activated by cyclins despite its structural similarity with other cyclin-dependent kinases. Rather, it is activated upon binding to activators p35 or p39. Cdk5 is ubiquitously expressed, but its activity is mostly limited to the nervous system due to the predominantly neural-specific expression of p35 and p39. Nonetheless, accumulating evidence demonstrates that Cdk5 activity can also be detected outside the nervous system, such as at the neuromuscular junction, pancreatic cells, adipose tissue and myeloid cells (Fu *et al.* 2001, Lilja *et al.* 2001, Choi *et al.* 2010, Arif *et al.* 2011). The importance of p35 and p39 as Cdk5 activators is further demonstrated by the comparable phenotype exhibited by Cdk5-deficient mice and p35/p39 double knock-out mice. Both exhibit perinatal death and severe cortical lamination defects (Ohshima *et al.* 1996, Ko *et al.* 2001). This is in contrast to single knock-out animals lacking p35 or p39, which survive through adulthood. In addition, aberrant cortical lamination was observed only in p35 knock-out mice (Ohshima *et al.* 1996, Ko *et al.* 2001, Chae *et al.* 1997). These observations indicate that while there is some redundancy, p35 and p39 are critical for the function of Cdk5.

2.1 Regulation of Cdk5 activity

Given the pivotal role of p35 and p39 in Cdk5 activation, changes in the expression level of p35 and p39 constitute one of the major mechanisms by which Cdk5 activity is regulated. Interestingly, Cdk5 activity is also regulated by calpain-mediated cleavage of p35 and p39 into p25 and p29, respectively. These fragments retain Cdk5-activating capability but exhibit significantly longer half-lives than p35 and p39, which are rather short-lived proteins (Patrick *et al.* 1999, Patzke and Tsai 2002). Cdk5 itself was found to contribute to the short half-life of p35 through phosphorylation of p35, which promotes its degradation by the proteasome pathway (Patrick *et al.* 1998). In addition, cleavage by calpain removes the myristoylation signal from p35 and p39, thus resulting in a redistribution of Cdk5 activity within the cell. Since p25 generation has been associated with excessive Cdk5 activation and neuronal loss in neurodegenerative disease (Patrick *et al.* 1999, Cruz and Tsai 2004), it has been speculated that Cdk5-p25 may be catalytically more active than Cdk5-p35. Nonetheless, a recent study demonstrated that the activity of Cdk5 is comparable regardless of whether it is associated with p35 or p25 (Peterson *et al.* 2010). These findings collectively suggest that the aberrant Cdk5 activity associated with p25 generation is likely due to prolonged activation of Cdk5, and not an elevated Cdk5 catalytic activity.

Aside from the regulation of p35 or p39 expression, and their degradation or cleavage, direct phosphorylation of Cdk5 also modulates its activity. Phosphorylation of Cdk5 at Tyr15 has been demonstrated to enhance Cdk5 activity (Sasaki *et al.* 2002, Fu *et al.* 2007, Cheung *et al.* 2007). This mechanism is particularly important for trophic factor-mediated regulation of Cdk5 activity, as a number of trophic factor receptors are receptor tyrosine kinases. Ligand binding triggers tyrosine kinase activation, which directly phosphorylates Cdk5 at Tyr15 to enhance Cdk5 activity. This phosphorylation was found to be crucial for the effect of Cdk5 on the signaling of trophic factors such as Ephrin A1 and BDNF (Cheung *et al.* 2007, Fu *et al.* 2007). Interestingly, S-nitrosylation of Cdk5 was also recently demonstrated to reduce Cdk5 activity (Zhang et al. 2010b). These findings indicate that post-translational modification of Cdk5 also constitutes an important mechanism for controlling Cdk5 activity.

2.2 Cdk5 as a regulator of neuronal survival

Despite being a member of the cyclin-dependent kinase family, Cdk5 is unique in several aspects. Not only is its mechanism of activation different, but its action in cell cycle regulation is also distinct from other cyclin-dependent kinases. Cyclin-dependent kinases are important enzymes that ensure the proper progression of cell cycle (Nguyen *et al.* 2002). The lack of Cdk5 expression in proliferating cells led to the conclusion that Cdk5 is not involved in the regulation of cell cycle progression (Tsai *et al.* 1993). Nonetheless, recent evidence indicates that Cdk5 also takes part in cell cycle control, acting as a suppressor of cell cycle re-entry in post-mitotic neurons (Zhang and Herrup 2008). Through the identification of a myriad of Cdk5 substrates, Cdk5 has been implicated in multiple aspects of neuronal development and neuronal functions. Aside from an obvious role of Cdk5 in neuronal migration as revealed by the severe cortical lamination defects in Cdk5-deficient mice (Ohshima *et al.* 1996), Cdk5 is also involved in neuronal differentiation and synapse formation during development (Cheung and Ip 2007). Furthermore, emerging evidence indicates that Cdk5 plays a critical role in synaptic function, synaptic plasticity and learning (Lai and Ip 2009, Hawasli and Bibb 2007).

The role of Cdk5 in neuronal survival is slightly more complex, and accumulating evidence suggests that Cdk5 functions as a double-edged sword. Following the identification of Cdk5 as a tau kinase (Kobayashi et al. 1993), deregulation of Cdk5 activity was found to contribute to neuronal loss in Alzheimer's disease (Patrick et al. 1999). Elevation of Cdk5 activity that is accompanied by p25 generation leads to neuronal death (Patrick et al. 1999). Although conflicting data have been obtained regarding the increase in p25 levels in post-mortem samples of Alzheimer's disease patients (Patrick *et al.* 1999, Li *et al.* 2003b, Tandon *et al.* 2003), subsequent studies have demonstrated augmented p25 expression and Cdk5 activity in response to a large number of pro-apoptotic agents or cell death stimuli, including MPTP (reviewed in Cheung and Ip 2004). These observations collectively establish a death-inducing role of Cdk5-p25. Interestingly, a pro-survival role of Cdk5 is also gaining recognition. Indeed, nuclear margination and cell swelling were observed in brainstem and spinal cord neurons in Cdk5-deficient brains (Ohshima et al. 1996). In addition, knock-down of Cdk5 expression alone results in apoptosis in developing retinal neurons (Cheung et al. 2008). Several mechanisms likely mediate the survival-maintaining property of Cdk5. For example, Cdk5 has been reported to be required for neuregulin-induced elevation of survival signaling pathway PI3K/Akt (Li et al. 2003a). In addition, phosphorylation of Bcl-2 at Ser70 by Cdk5 is essential for its anti-apoptotic property, which is pivotal for the maintenance of neuronal survival during development (Cheung et al. 2008). Cdk5 has also been demonstrated to exhibit a neuroprotective role through suppression of cell cycle re-entry, which is associated with cell death in post-mitotic neurons (Zhang et al. 2010a).

How Cdk5 manages to mediate both cell death and survival signals is incompletely understood. It is generally believed that while basal level of Cdk5 activity is required for neuronal survival, excessive activation, particularly in the presence of p25, leads to neuronal death. In addition, recent studies suggest that the subcellular localization of Cdk5 activity may also determine whether it serves a protective or detrimental role. Cdk5 activity has been demonstrated in the cytoplasm and nucleus. Myristoylation of p35 and p39 has been shown to regulate the distribution of Cdk5, with non-myristoylated p35 and p39 preferentially accumulated in the nucleus (Asada et al. 2008). Previously it has been suggested that nuclear Cdk5 may be selectively associated with neuronal loss, while cytoplasmic Cdk5 activity is linked to neuroprotection (O'Hare et al. 2005). Nonetheless, recent studies indicate that both cytoplasmic and nuclear Cdk5 activity can contribute to neuronal loss (Rashidian et al. 2009) or neuronal survival (Zhang et al. 2010a). It appears that the differential distribution of Cdk5 in the cytoplasm and nucleus, and their respective functions, depend on the types of insults that are being inflicted. Additional studies will be required to further delineate the precise involvement of nuclear and cytoplasmic Cdk5 in the regulation of neuronal survival.

3. Cdk5 in PD pathology

In agreement with the essential role of Cdk5 as a regulator of neuronal survival, Cdk5 is also implicated in neuronal loss in PD, with elevation of Cdk5 consistently associated with cell death in different PD models. Cdk5 was first linked to PD when inhibition of Cdk5 activity reduces neuronal loss in MPTP-injected mice (Smith et al. 2003). MPTP injection increases Cdk5 activity and p25 levels in the substantia nigra of the injected mice, and attenuating this increase with Cdk5 inhibitor or adenovirus-mediated overexpression of dominant-negative Cdk5 attenuates dopaminergic neuronal loss (Smith et al. 2003). Although treatment with

MPP^+, the metabolized form of MPTP in the brain, has also been demonstrated to reduce Cdk5 activity through proteasome-dependent degradation of p35 (Endo et al. 2009), majority of the studies reported increase in Cdk5 activity following MPTP injection or MPP^+ treatment (Smith *et al.* 2003, Wong *et al.* 2011, Smith *et al.* 2006, Qu *et al.* 2007, Huang *et al.* 2010), possibly due to the use of different toxin dosages. Interestingly, additional studies aimed at elucidating the mechanisms by which Cdk5 regulates neuronal loss implicate Cdk5 in multiple cellular processes that were found to be altered in PD. Here we summarize the role of Cdk5 in several pathogenic mechanisms postulated to contribute to PD pathology.

3.1 Autophagy deregulation

Autophagy, a homeostatic process for the turnover of cytoplasmic content and organelles, is increasingly implicated in neurodegenerative diseases. Currently three types of autophagy are identified based on the mechanisms by which cargo is delivered, namely macroautophagy, microautophagy and chaperone-mediate autophagy (CMA). Macroautophagy is initiated with the formation of autophagosome, a double-membraned vesicle that is formed through the extension of isolation membrane (also known as phagophore), to encircle part of the cytoplasm for degradation. Subsequent fusion of autophagosome with lysosome leads to the formation of autolysosome, where the cargo of the autophagosome is degraded by lysosomal enzymes (Mizushima 2007). Microautophagy also entails bulk sequestration of cytoplasmic content, but instead of acting through the formation of autophagosomes, it is directly sequestered into lysosomes. CMA, on the other hand, involves selective translocation of soluble target proteins, which usually contain the KFERQ motif, to the lysosomes. Heat-shock cognate 70 (hsc70) and lysosomes-associated membrane protein 2A (LAMP2A) are important chaperones for selective transport of cargo into the CMA pathway (Cheung and Ip 2009, Mizushima *et al.* 2008, Rubinsztein 2006).

While autophagy has long been regarded as a homeostatic cellular event, recent studies revealed that deregulation of the autophagic pathway also contributes to neurodegeneration. Transgenic animals lacking Atg5 or Atg7, genes that are critical for macroautophagy, develop neurodegeneration (Hara *et al.* 2006, Komatsu *et al.* 2006). In addition, activation of macroautophagy in a drosophila model of Huntington's disease significantly reduces huntingtin toxicity (Ravikumar et al. 2004), consistent with a role of macroautophagy in the clearance of protein aggregates. Deregulation of the autophagic pathway is also demonstrated in PD (Cheung and Ip 2009). For example, accumulation of autophagosomes is evident in post-mortem brains of PD patients (Mizushima et al. 2008). In addition, both CMA and macroautophagy were implicated in the regulation of α-synuclein level, the major constituent of Lewy body. In particular, CMA is required for the degradation of wildtype soluble α-synuclein (Cuervo *et al.* 2004, Vogiatzi *et al.* 2008, Webb *et al.* 2003). Interestingly, A53T and A30P mutants of α-synuclein, which are associated with familial PD, inhibit CMA-mediated degradation of wildtype α-synuclein. These mutants are in turn degraded by macroautophagy (Cuervo et al. 2004). Furthermore, inhibition of CMA by A53T α-synuclein mutant also impairs degradation of pro-survival transcription factor MEF2D (Yang et al. 2009). Reduced degradation of MEF2D results in accumulation of MEF2D in the cytoplasm and inhibition of MEF2D activity, leading to cell death (Yang et al. 2009). Interestingly, Cdk5 has been demonstrated to phosphorylate MEF2D at S444 to inhibit its activity and facilitate its cleavage by caspases (Tang *et al.* 2005, Gong *et al.* 2003), and is required for neuronal loss triggered by MPTP injection (Smith et al. 2006). It will be

interesting to examine whether phosphorylation of MEF2D by Cdk5 plays a role in its degradation by CMA. Moreover, we have recently demonstrated that overexpression of A53T α-synuclein increases Cdk5 activity (Wong et al. 2011). It is tempting to speculate that in addition to the inhibition of CMA-mediated degradation of MEF2D by α-synuclein expression, elevated phosphorylation of MEF2D by Cdk5 may also contribute to cell loss triggered by α-synuclein expression. Studies aimed at addressing this possibility will provide important insights regarding the pathogenic mechanisms of PD.

It should be noted that overexpression of A53T and A30P mutants of α-synuclein also induces activation of macroautophagy (Wong *et al.* 2011, Cuervo *et al.* 2004, Vogiatzi *et al.* 2008). While induction of macroautophagy may facilitate clearance of these mutant α-synucleins, several studies suggest that elevation of macroautophagy in response to A53T α-synuclein expression or MPP^+ treatment may be associated with neuronal loss (Wong *et al.* 2011, Yang *et al.* 2009, Xilouri *et al.* 2009, Stefanis *et al.* 2001, Kirik *et al.* 2002, Choubey *et al.* 2011). Whether this induction serves a protective or detrimental role in PD thus remains unresolved. We have recently discovered a role of Cdk5 in the regulation of macroautophagy through its phosphorylation of lipid-binding protein endophilin B1 (Wong et al. 2011). Endophilin B1, also known as Bax-interacting factor 1 (Bif-1), was previously implicated in autophagy induction in fibroblasts through its association with autophagy machinery UVRAG and Beclin 1 (Takahashi et al. 2007). We demonstrated that Cdk5-mediated phosphorylation of endophilin B1 at T145 is required for starvation-induced macroautophagy in neurons. More importantly, our findings revealed that this phosphorylation event is critical for the activation of macroautophagy in response to MPP^+ stimulation or overexpression of A53T α-synuclein (Wong et al. 2011). Attenuation of macroautophagy induction significantly reduces neuronal loss in these PD models, suggesting that activation of macroautophagy in PD may contribute to neuronal loss. Remarkably, inhibition of Cdk5 activity or endophilin B1 T145 phosphorylation concomitantly reduces macroautophagy activation or neuronal loss (Wong et al. 2011). Collectively, our findings reveal that macroautophagy activation in PD may serve a detrimental role, with Cdk5 and endophilin B1 being the essential mediators of this induction.

Deregulation of autophagy in PD also occurs in the form of impaired mitophagy, the removal of mitochondria through macroautophagy (Youle and Narendra 2011). Recent evidence reveals that two genes that are mutated in cases of familial PD, PINK1 and Parkin, are critical for mitophagy (Narendra *et al.* 2008, Geisler *et al.* 2010, Lee *et al.* 2010, Jin *et al.* 2010, Ziviani *et al.* 2010). PINK1, a serine/threonine kinase that is expressed on the outer membrane of the mitochondria, is constitutively degraded in healthy mitochondria in a voltage-dependent manner. The high level of PINK1 on damaged mitochondria will then result in the recruitment of Parkin, a ubiquitin E3 ligase, to the mitochondria. Subsequent ubiquitination of the damage mitochondria, Parkin itself and VDAC is required for the removal of damaged mitochondria via mitophagy (Narendra *et al.* 2010, Geisler *et al.* 2010, Lee *et al.* 2010, Jin *et al.* 2010). Importantly, various PD-associated missense mutations have been demonstrated to trigger different extent of mitophagy impairment (Lee et al. 2010), suggesting that aberrant mitophagy may represent one of the mechanisms by which these mutations lead to PD. Interestingly, Cdk5 has been demonstrated to phosphorylate Parkin at S131 to reduce its E3 ubiquitin ligase activity, with the S131A mutant of Parkin more prone to accumulate into inclusions (Avraham et al. 2007). In addition, phosphorylation of Parkin

by Cdk5 and casein kinase I also augments its aggregation and inactivation (Rubio de la Torre et al. 2009). It will be interesting to examine whether Cdk5-mediated inhibition of Parkin activity affects the mitophagy of damaged mitochondria in PD models.

3.2 Oxidative stress

Oxidative stress has long been demonstrated to play an essential role in PD pathogenesis. Lipid peroxidation, DNA damage and protein oxidation are all evident in PD brains (Levy et al. 2009). In support of the involvement of oxidative stress in PD, familial PD-associated missense mutations were identified in DJ-1, an atypical peroxiredoxin-like peroxidase with antioxidant activity (Andres-Mateos et al. 2007). In addition, mitochondrial dysfunction, which could directly lead to generation of reactive oxygen species, is also detected in PD. These findings collectively suggest that preserving the anti-oxidative machinery in PD will be critical for limiting PD pathology.

Interestingly, an anti-oxidative peroxidase peroxiredoxin 2 (Prx2) was found to be phosphorylated by Cdk5 in response to MPTP toxicity (Qu et al. 2007). Phosphorylation of Prx2 at T89 by Cdk5 reduces its peroxidase activity. In addition, treatment with MPP^+ increases Cdk5 activity and phospho-T89 Prx2 level, while concomitantly decreasing Prx2 peroxidase activity. Importantly, the protective effect of Prx2 against MPP^+-induced cell death is attenuated when a T89 phospho-mimetic mutant of Prx2 is expressed, suggesting that phosphorylation of Prx2 at T89 abrogates its protective effect (Qu et al. 2007). These observations collectively revealed that Cdk5 may contribute to neuronal loss in PD through inhibiting the peroxidase activity of Prx2, thus rendering the cells more susceptible to oxidative stress.

DNA damage is a frequent consequence of oxidative stress and is a known factor for triggering cell death. A recent study revealed that Cdk5 also mediates neuronal loss in PD through phosphorylation of apurinic/apyrimidinic endonuclease 1 (Ape1), an enzyme implicated in DNA repair (Huang et al. 2010). Cdk5-mediated phosphorylation of Ape1 at T232 reduces its endonuclease activity and abolishes its neuroprotective effect against MPP^+-triggered neuronal death (Huang et al. 2010). Interestingly, another enzyme implicated in DNA damage response, ataxia telangiectasia mutated **(**ATM), is also identified as a Cdk5 substrate (Tian et al. 2009). ATM is a phosphoinositide-3-kinase related kinase that plays critical role in the mediation of DNA damage signals, and has been implicated in neuronal loss triggered by DNA damage (Herzog *et al.* 1998, Kruman *et al.* 2004, Lee *et al.* 2001). Elevation of Cdk5 activity in response to DNA damage results in phosphorylation of ATM at S1981, an event that is critical for the activation of ATM. Interestingly, inhibition of Cdk5 activity attenuates neuronal loss induced by DNA damaging agent camptothecin (Tian et al. 2009). Although whether ATM phosphorylation is triggered in PD models remains unexplored, given the implication of DNA damage and the potential induction of Cdk5 activity in PD, it will be interesting to further dissect the precise role of Cdk5 in regulating the anti-oxidative machinery of the cells.

3.3 Mitochondrial dysfunction

Mitochondrial dysfunction has emerged as an important pathogenic mechanism in PD. Reduced mitochondrial complex I activity was detected in PD patients (Levy et al. 2009). In addition, MPTP was demonstrated to inhibit complex I of the mitochondrial respiratory chain (Levy et al. 2009). More importantly, gene products of a number of the familial PD-

associated missense mutations are found to be localized to the mitochondria, including PINK1, Parkin, LRRK2, DJ-1 and HtrA2/Omi (Knott et al. 2008). In particular, recent studies revealed PINK1 and Parkin as important regulators of mitochondrial morphology through facilitating mitochondrial fission (Cho et al. 2010). Given the association of neuronal apoptosis with elevated mitochondrial fission, regulation of mitochondrial fusion/fission event by PINK1 and Parkin may also contribute to neuronal loss in PD. In addition, as mentioned above, PINK1 and Parkin are also required for the selective mitophagy of mitochondria with low membrane potential (Youle and Narendra 2011). With Parkin identified as a Cdk5 substrate (Avraham et al. 2007) and the observed expression of Cdk5 and p35 in the mitochondrial fraction of cortical neurons (Cheung et al. 2008), it is tempting to speculate that Cdk5 may also regulate mitochondrial homeostasis during PD through controlling mitochondrial fission/fusion or mitophagy. In support of this possibility, Cdk5 has also been implicated in mitochondrial fission during apoptosis (Meuer et al. 2007). In addition, another Cdk5 substrate endophilin B1 is also demonstrated to regulate mitochondrial morphology (Wong *et al.* 2011, Karbowski *et al.* 2004). It is therefore important to further delineate the role of Cdk5 in mitochondrial morphogenesis and mitophagy in PD models.

Aside from being the powerhouse of the cell and a potential source of reactive oxygen species, the mitochondria also plays a central role in the intrinsic apoptotic pathway. Release of cytochrome c from the intermembrane space of mitochondria triggers caspase activation and apoptosis (Bredesen et al. 2006). Indeed, neuronal loss in PD has been attributed at least in part to the apoptotic pathway (Levy et al. 2009). Identification of Bcl-2, a key anti-apoptotic regulator of cytochrome c release from the mitochondria, as a Cdk5 substrate suggests that Cdk5 may also regulate neuronal loss in PD through phosphorylation of Bcl-2 (Cheung et al. 2008). Furthermore, Cdk5 substrate endophilin B1, being an interacting protein of pro-apoptotic Bcl-2 family member Bax (Wong *et al.* 2011, Cuddeback *et al.* 2001), has also been demonstrated to play a role in apoptosis. Inhibition of endophilin B1 expression attenuates cytochrome c release and caspase-3 activation in HeLa cells (Takahashi et al. 2005). Although whether endophilin B1 similarly regulates apoptosis in neurons remains to be explored, these observations suggest that Cdk5-mediated phosphorylation of endophilin B1 and Bcl-2 may regulate the mitochondrial apoptotic pathway in PD. Additional studies will be required to address this hypothesis.

3.4 Proteasomal pathway anomaly

The proteasome pathway has also been demonstrated to contribute to degradation of α-synuclein (Webb *et al.* 2003, Rubinsztein 2006), suggesting that reduction in proteasomal activity may contribute to α-synuclein aggregation. Strikingly, a 40% decrease in proteasome activity has been demonstrated in the substantia nigra of PD patients post-mortem brains (McNaught and Jenner 2001). Indeed, mutation in E3 ubiquitin ligase Parkin and ubiquitin-C-terminal hydrolase-L1 (UCH-L1), a protein implicated in the degradation of poly-ubiquitin chains, are evident in familial PD cases (Kitada *et al.* 1998, Leroy *et al.* 1998). Familial PD-associated missense mutation of Parkin reduces its enzyme activity (Shimura et al. 2000). With Cdk5-mediated phosphorylation of Parkin also demonstrated to reduce its E3 ubiquitin ligase activity, it will be important to investigate if Cdk5 is involved in the deregulation of the proteasomal pathway in PD.

4. Future perspectives

It has become increasingly clear that Cdk5 plays a pivotal role in neuronal loss in PD. Using both *in vivo* and *in vitro* cell-based systems, inhibition of Cdk5 consistently attenuates neuronal loss in various PD models. The identification of multiple Cdk5 substrates that directly participates in known pathogenic mechanisms of PD has provided the much needed mechanistic insights regarding the alteration of these processes in PD, and the precise involvement of Cdk5 (Figure 1). Nonetheless, our understanding is far from complete. In particular, in light of the convergence of several pathogenic mechanisms onto the mitochondria and the identification of a number of mitochondria-associated proteins as Cdk5 substrates, it is important to further delineate how Cdk5 regulates the signaling cascades at the mitochondria during PD. In addition, Cdk5 has also been demonstrated to

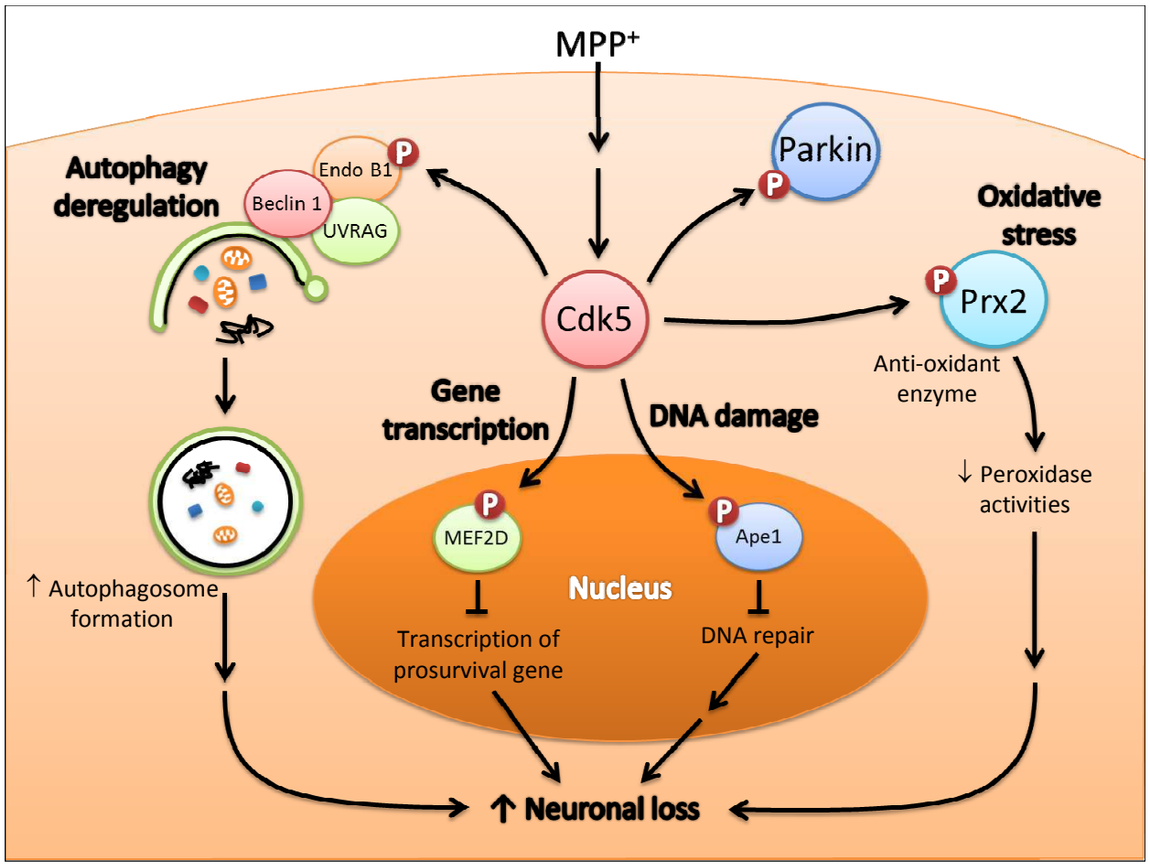

Fig. 1. Implication of Cdk5 in neuronal loss in PD through phosphorylation of multiple substrates. MPTP injection or MPP+ treatment elevates Cdk5 activity, which in turn phosphorylates a number of cellular proteins. Phosphorylation of endophilin B1 by Cdk5 is required for macroautophagy induction in PD models, which contributes to neuronal loss. Cdk5 also phosphorylates pro-survival transcription factor MEF2D and DNA repair enzyme Ape1 to inhibit their activities, thereby leading to cell death. Phosphorylation of anti-oxidant enzyme Prx2 by Cdk5 attenuates its peroxidase activity, leaving the cell more prone to oxidative stress. Cdk5-mediated phosphorylation of Parkin also reduces its E3 ubiquitin ligase activity, but how this directly contributes to neuronal loss remains to be explored.

regulate dopaminergic transmission and dopamine downstream signaling through phosphorylation of DARPP-32 (Chergui *et al.* 2004, Bibb *et al.* 1999). Furthermore, both dopamine and tyrosine hydroxylase have been identified as Cdk5 substrates (Zhen *et al.* 2004, Kansy *et al.* 2004). It will thus be important to further examine the effect of Cdk5 on dopaminergic transmission in PD models, and investigate the possibility that Cdk5 modulators may regulate dopamine levels to alleviate PD symptoms. Together with the apparent neuroprotective effect of Cdk5 against neuronal loss in PD through acting on multiple cellular events that are aberrant in PD, Cdk5 may emerge as an important target for future development of therapeutics against PD.

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Parkinson's disease (PD) results primarily from the death of dopaminergic neurons in the substantia nigra. Current PD medications treat symptoms; none halt or retard dopaminergic neuron degeneration. The main obstacle to developing neuroprotective therapies is a limited understanding of the key molecular mechanisms that provoke neurodegeneration. The discovery of PD genes has led to the hypothesis that misfolding of proteins and dysfunction of the ubiquitin-proteasome pathway are pivotal to PD pathogenesis. Previously implicated culprits in PD neurodegeneration, mitochondrial dysfunction, and oxidative stress may also act in part by causing the accumulation of misfolded proteins, in addition to producing other deleterious events in dopaminergic neurons. Neurotoxin-based models have been important in elucidating the molecular cascade of cell death in dopaminergic neurons. PD models based on the manipulation of PD genes should prove valuable in elucidating important aspects of the disease, such as selective vulnerability of substantia nigra dopaminergic neurons to the degenerative process.

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