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Novel Cell Death Signaling Pathways in Neurotoxicity Models of Dopaminergic Degeneration: Relevance to Oxidative Stress and Neuroinflammation in Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a common neurodegenerative movement disorder characterized by extensive degeneration of dopaminergic neurons in the nigrostriatal system. Neurochemical and neuropathological analyses clearly indicate that oxidative stress, mitochondrial dysfunction, neuroinflammation and impairment of the ubiquitin-proteasome system (UPS) are major mechanisms of dopaminergic degeneration. Evidence from experimental models and postmortem PD brain tissues demonstrates that apoptotic cell death is the common final pathway responsible for selective and irreversible loss of nigral dopaminergic neurons. Epidemiological studies imply both environmental neurotoxicants and genetic predisposition are risk factors for PD, though the cellular mechanisms underlying selective dopaminergic degeneration remain unclear. Recent progress in signal transduction research is beginning to unravel the complex mechanisms governing dopaminergic degeneration. During 12th International Neurotoxicology meeting, discussion at one symposium focused on several key signaling pathways of dopaminergic degeneration. This review summarizes two novel signaling pathways of nigral dopaminergic degeneration that have been elucidated using neurotoxicity models of PD. Dr. Anumantha Kanthasamy described a cell death pathway involving the novel protein kinase C delta isoform (PKC δ) in oxidative stress-induced apoptotic cell death in experimental models of PD. Dr. Ajay Rana presented his recent work on the role of mixed lineage kinase-3 (MLK3) in neuroinflammatory processes in neurotoxic cell death. Collectively, PKC δ and MLK3 signaling pathways provide new understanding of neurodegenerative processes in PD, and further exploration of these pathways may translate into effective neuroprotective drugs for the treatment of PD.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Keywords

Protein kinase C δ ; MLK3; Parkinson's disease; neurotoxicity; Dieldrin; Manganese; environmental factors; Translational research

1.0 Role of PKC δ Signaling in Neurotoxicity Models of Parkinson's Disease (A.G.K)

Parkinson's disease (PD) is a devastating neurodegenerative disorder affecting several million people worldwide. It inflicts a tremendous social and economic burden on modern society where the incidence of the disease increases with age. Currently, the mean age of onset is around 55 years. In all cases, the clinical features which characterize PD, including resting tremor, bradykinesia, and postural instability (Fahn and Sulzer, 2004), are progressive. Distinct among the pathological features of PD is the significant loss of dopaminergic neurons in the substantia nigra leading to a dramatic depletion of dopamine in the striatum. The current PD treatment approach employing levodopa, an intermediate molecule in the genesis of dopamine, is ranked among the most remarkable success stories in medical history. However, the drug confers only symptomatic relief of what remains an inexorably progressive and ultimately fatal neurodegenerative disorder. As a result, the need for novel neuroprotective agents designed to interfere with the basic pathogenic mechanism of cell death in PD are clearly needed.

1.1 Environmental risk factors and Parkinson's disease

The specific etiology of PD is still elusive, although results from extensive studies reveal that both accumulated environmental toxicant exposure and genetic mutations contribute to the onset of PD (Thomas, 2009)(Gerlach and Riederer, 1999). While sporadic cases of PD are largely attributed to genetic causes (approximately 90 percent), environmental toxin exposure triggering neuronal apoptosis is considered a dominant risk factor in the development of this disease. Most compelling among this evidence was the discovery of a synthetic heroin analog, 1,2,3,6-methyl -phenyl-tetrahydropyridine (MPTP), that was noted to produce a syndrome clinically and pathologically resembling PD in a group of narcotics addicts (Langston et al., 1983). The neurotoxicology of MPTP has subsequently been well characterized; MPP⁺, the active metabolite of MPTP, enters dopaminergic neurons via a dopamine transporter and inhibits the complex I of the mitochondrial respiratory chain, thereby selectively causing toxicity to dopaminergic neurons (Nicklas et al., 1985). In addition to MPTP, a variety of additional exogenous or endogenous toxic agents have been recognized to cause PD-like syndromes including dopamine and its metabolites, certain metals, and several agricultural chemicals (Kanthasamy et al., 2005). Many epidemiological, case-control and postmortem studies provide evidence for the involvement of heavy metals in PD pathogenesis (Aschner et al., 2009; Uversky et al., 2001). Of these metals, manganese (Mn) gains more attention than others because of its role in the human neurological condition known as manganism, which is characterized by clinical signs and morphological lesions similar to those seen in PD. Despite the similarities between manganism and PD, some distinct clinical and pathological differences are evident between the diseases. The cellular mechanisms underlying the neurotoxicology of manganese are still unclear although accumulating evidence indicates that neuronal apoptosis resulting from oxidative stress and mitochondrial dysfunction may play an important role. With regard to agricultural chemicals and PD etiology, dieldrin, an organochlorine pesticide, has been associated with PD based on postmortem and epidemiological studies (Kanthasamy et al., 2005; Richardson et al., 2006). Postmortem analysis revealed that dieldrin was present in PD brains, but not in control brains (Fleming et al., 1994). Moreover, a case-control study also demonstrated that dieldrin was a risk factor in PD (Semchuk et al., 1991).

1.2 Involvement of apoptosis in Parkinson's disease

Apoptosis or programmed cell death is a genetically regulated death process of cells involving caspase activation and a lack of cell swelling (Williams and Smith, 1993). Apoptosis is recognized as a key fundamental and indispensable process in any normal cell function. Aberrant regulation of apoptosis is a common feature in many diseases including neurodegenerative diseases and cancer and is now widely accepted as a crucial cause of dopaminergic neuronal death in PD. This is based on extensive postmortem analysis of PD brains as well as experimental models (Anglade et al., 1997; Jellinger and Stadelmann, 2000; Kaul et al., 2003; Mochizuki et al., 1996; Tatton and Kish, 1997; Yuan and Yankner, 2000). Induction of apoptosis by both environmental insults and PD genetic predisposition suggests that biochemical events involved in the cell death process are highly conserved despite the differences in the nature of neurotoxic insults.

Impairment of mitochondrial function by neurotoxicants is known to result in the selective degeneration of dopaminergic neurons (Kanthasamy et al., 1994; Pallanck and Greenamyre, 2006; Schapira, 2008; Winkhofer and Haass, 2009). We and others have shown that neurotoxins such as MPP⁺ (Kaul et al., 2003), dieldrin (Kitazawa et al., 2004), manganese (Latchoumycandane et al., 2005), rotenone (Sherer et al., 2003; Testa et al., 2005), and paraquat (McCormack et al., 2005) activate major events of the apoptotic pathway, including cytochrome C release, caspase-3 activation, and DNA fragmentation. Thus, apoptosis is the primary cell death process of dopaminergic neurons and therefore, understanding the molecular mechanisms of apoptosis continues to be an important area of investigation in neurotoxicology.

1.3 Protein kinase C delta (PKC δ) and neuronal apoptosis

The detailed understanding of the molecular and cellular mechanisms in neuronal apoptosis offer more promising points of entry into the therapeutics of PD. Already, many signaling pathways have been identified in the neurodegenerative processes of dopaminergic neurons. During the past several years, our laboratory has focused on identifying key players involved in dopaminergic neuronal apoptosis and we found that protein kinase C delta (PKC δ) is an oxidative stress-sensitive kinase, which functions as a key mediator in apoptotic cell death in PD (Anantharam et al., 2002; Kanthasamy et al., 2003; Kaul et al., 2005; Kaul et al., 2003; Latchoumycandane et al., 2005; Yang et al., 2004). We also have developed translational PD therapeutic strategies targeting the proapoptotic PKC δ cell death signaling pathway (Kanthasamy et al., 2006; Zhang et al., 2007a). At least 12 isoforms have been identified and further divided into three groups: conventional PKCs (α , β I, β II, γ), novel PKCs (δ , ϵ , η , θ), and atypical PKCs (ζ , ι , λ), each based on its lipid requirement and dependency on Ca²⁺ for activation. PKC δ was first discovered by Gschwendt et al. (Gschwendt et al., 1986), and belongs to the protein kinase C serine/threonine kinase family. This family of kinase plays a role in the regulation of multiple cellular responses, including proliferation, cell cycle progression, differentiation, survival, and apoptosis (Dempsey et al., 2000; Kanthasamy et al., 2003). PKC δ , a Ca²⁺-independent isoform, is ubiquitously expressed in most tissues including brain, spleen, ovary, lung and uterus (Leibersperger et al., 1991). A survey of expression of PKC isoforms in the rat brain indicates that PKC δ is highly expressed in the thalamus, septal nuclei, and hippocampal CA1 pyramidal cell layer (Naik et al., 2000). Recently, we conducted double immunostaining analysis and confocal microscopy to evaluate the expression of PKC δ in nigral dopaminergic neurons (Zhang et al., 2007b). Our results revealed that PKC δ is highly expressed in mouse nigral tissues, and more importantly, PKC δ co-localizes with tyrosine hydroxylase (TH) in the substantia nigra. Also, we showed that PKC δ negatively regulates TH activity and dopamine synthesis, demonstrating an anatomical and functional relationship of the kinase in the nigrostriatal pathway (Zhang et al., 2007b).

The domain structure of PKC δ is presented in Fig. 1. Consistent with other PKC isoforms, PKC δ consists of a regulatory domain (N-terminus) and a catalytic domain (C-terminus). Like other conventional and novel PKC isoforms, PKC δ is primarily activated by a lipid-mediated mechanism involving its translocation from cytosol to membrane. Two other pathways of PKC δ activation also have been elucidated: phosphorylation and proteolytic activation (Brodie and Blumberg, 2003; Kanthasamy et al., 2003; Kikkawa et al., 2002). Reportedly, phosphorylation of Thr-505, Ser-643, and Ser-662 in the activation loop can increase its kinase activity (Toker, 1998). In addition to the phosphorylation of Thr/Ser sites, tyrosine phosphorylation at tyrosine residues Tyr-52, Tyr-155, Tyr-187, Tyr-311, Tyr-332, and Tyr-565 has also been implicated in modulating activity (Gschwendt, 1999) in various cell types. Various types of stimulation reportedly induce the tyrosine phosphorylation of PKC δ (Kikkawa et al., 2002). For example, treatment with the known oxidative stress-inducing agent hydrogen peroxide (H₂O₂) reportedly caused Tyr-311 and Tyr-332 phosphorylation of PKC δ (Konishi et al., 2001). We have found that under certain stimulation, e.g., H₂O₂, the phosphorylation of Tyr-311 on PKC δ is particularly important for the proteolytic activation of PKC δ in dopaminergic neurons (Kaul et al., 2005). Because multiple tyrosine residues on PKC δ can be phosphorylated by upstream kinase, the effect of tyrosine phosphorylation may be different, depending on both the position of the phosphorylated tyrosine and the specific cellular context. We and others have identified an additional proteolytic activation mechanism of PKC δ that results in catalytic and regulatory fragments due to proteolysis. The cleavage of PKC δ is mediated by caspase-3, resulting in 41-kDa catalytically active and 38-kDa regulatory fragments. Compared to membrane translocation and serine/tyrosine phosphorylation, proteolytic cleavage of PKC δ causes a persistent activation of the kinase. Importantly, PKC δ proteolytic activation mediates apoptotic cell death.

1.4 PKC δ proteolytic activation in neurotoxicity models of dopaminergic degeneration

The proteolytic activation of PKC δ has been implicated in apoptosis in many cell types (Choi et al., 2006; D'Costa and Denning, 2005; Ryer et al., 2005). Our recent studies have characterized a critical role for the caspase-3-dependent proteolytic activation of PKC δ in oxidative stress-induced dopaminergic cell death in cell culture models of PD. In rat mesencephalic dopaminergic neuronal N27 cell models, a dose-dependent and time-dependent increase in the proteolytic activation of PKC δ was identified following exposure to dopaminergic neurotoxins such as inorganic manganese (Latchoumycandane et al., 2005), the organic manganese found in the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT) (Anantharam et al., 2002), the agriculture chemical dieldrin (Kitazawa et al., 2003), MPP⁺ (Kaul et al., 2003; Yang et al., 2004), the proteasome inhibitor MG-132 (Sun et al., 2008), or the oxidative stress inducing agent H₂O₂ (Kaul et al., 2005). We also found that the active PKC δ isoform was not translocated to the cell membrane during neurotoxic insults, suggesting that the lipid-mediated activation mechanism is not involved in this process. Figure 2 shows PKC δ cleavage in N27 cells increased after manganese, dieldrin, or MG-132 treatment. Furthermore, using both pharmacological inhibitors (PKC δ specific inhibitor rottlerin; and caspase-3 inhibitors z-DEVD-fmk or z-DIPD-fmk) and genetic tools (PKC δ siRNA or a PKC δ cleavage-resistant mutant), we demonstrated that the caspase-3 dependent proteolytic activation of PKC δ plays an important role in neurotoxicant-induced apoptotic death (Kanthasamy et al., 2006; Sun et al., 2008; Yang et al., 2004). As shown in Figure 3, the caspase-3 inhibitor z-DEVD-fmk and the PKC δ cleavage site peptide inhibitor z-DIPD-fmk effectively blocked the Parkinsonian toxicant MPP⁺ (300 μ M) and the prooxidant H₂O₂ (100 μ M) induced PKC δ cleavage. We demonstrated that the PKC δ cleavage site peptide inhibitor z-DIPD-fmk was more potent than the general caspase-3 inhibitor z-DEVD-fmk in protecting dopaminergic neurons against apoptotic cell death, suggesting the possibility of a novel neuroprotective strategy targeting PKC δ proteolytic activation. We have noted that the proteolytically cleaved PKC δ catalytic fragment translocates into the nucleus. In the nucleus,

PKC δ can induce phosphorylation of lamin B (Cross et al., 2000). Several other proteins also interact with PKC δ , including DNA-dependent protein kinase (DNA-PK) (Bharti et al., 1998) and p73 (Ren et al., 2002). Additionally, a positive feedback amplification loop between PKC δ and caspases-3 has been discovered by our laboratory. We found that the proteolytic activation of PKC δ regulates upstream caspase-3 activity, thus suggesting that PKC δ may function as both a mediator and signal amplifier during the neurotoxin-induced apoptotic pathway.

2.0 Mixed Lineage kinase-3 Signaling: Relevance to Neuroinflammatory Processes in Neurotoxic Cell Death (AR)

2.1 Mixed Lineage Kinases (MLKs) and neuronal apoptosis

For the last several years our laboratory has focused on the dissection of the detailed cell signaling network mediated via a relatively novel and new family of kinases called Mixed Lineage Kinases (MLK). MLK are unique in that all family members contain signature sequences of Ser/Thr and Tyr kinases within their catalytic domains and thus they are called hybrid or mixed kinases. We have shown that MLK3, a member of MLK family, acts as MAPKKK and specifically activates the jun-N-terminal kinase (JNK) (Figure 4A) (Rana et al., 1996). In the JNK pathway, the MAP3K members, like mixed lineage kinases (MLKs), phosphorylate MAP2K members SEK1/MKK4 and MKK7 (Rana et al., 1996; Gallo et al., 2002). The activated MAP2K subsequently phosphorylate JNK at the tyrosine and threonine residues leading to phosphorylation and activation of transcription factors such as c-Jun, ELK-1 and ATF-2. The role of MLK in neuronal cell death pathways was unknown until recently when a specific inhibitor of MLK family, CEP-1347, and its analogue CEP-11004 were identified (Maroney et al., 2001). Subsequently, it was shown that a CEP compound appeared to prevent dopaminergic neuronal cell death in an MPTP model of Parkinson's disease (PD) (Saporito et al., 1999; Teismann et al., 2003). Whether the mediated dopaminergic neuronal loss observed in this model of PD resulted from JNK activation or from an unknown pathway is still not established. However, it has been reported in studies of several cell culture models that the activation of JNK promotes cell death (Davis, 2000; Kyriakis et al., 2001) and therefore it is expected that activation of the MLK-JNK pathway also results in neuronal cell death. The CEP compounds capable of inhibiting MLK activation are not specific to a particular isoform and therefore in vivo studies with these inhibitors do not implicate specific MLK members in dopaminergic cell loss. The involvement of a specific MLK isoform can be identified only by generating genetic mouse models, where specific MLK members can be ablated. Interestingly, MLK3-and MLK1+MLK2-compound knockout mice have been generated recently (Brancho et al., 2005; Bisson et al., 2008). However, their role in dopaminergic neuronal loss has not been investigated. Currently investigation is underway in our lab to identify the specific MLK isoform capable of mediating dopaminergic neuronal loss in MPTP mouse models of PD.

2.2 Neuro inflammation and MLKs-mediated cell death

Neuroinflammation has been reported to cause dopaminergic neuronal cell death in PD patients (Hald et al., 2005). Elevated levels of neuroinflammatory cytokines such as TNF- α , have been detected in animal model of PD and human postmortem brain samples from PD (Dufek et al., 2009). While specific agonists for each MLK member previously were not known, we recently identified TNF- α and ceramide as specific agonists of MLK3 (Figure 4B) (Sathyanarayana et al., 2002). In this study, we showed that MLK3 and its downstream target, JNK, were activated potently by TNF- α and ceramides (Sathyanarayana et al., 2002). The TNF- α is a proinflammatory cytokine implicated in the cell death pathway (Tansey et al., 2008). While it is possible that these proinflammatory pathways impinge on MLK3-JNK activation and promote dopaminergic neuronal cell death, elevated levels of circulatory ceramides have also been reported in PD patients (Arboleda et al., 2009). Similar to proinflammatory cytokine TNF-

α , ceramides are also pleiotropic in nature having a range of physiological effects including the inducement of cell death (Villena et al., 2008). The premise that TNF- α and ceramides, both agonists of MLK3, might promote dopaminergic cell death via MLK3, is currently being examined in our lab via MLK3 knockout mice. Additionally, it is possible that TNF- α itself is regulated by the MLK3-JNK pathway because the TNF- α promoter contains AP-1 binding sites, which are ultimately regulated by JNK activation.

2. 3 Regulation of MLK by upstream kinases and its implication in neuronal cell death

Dopaminergic neuronal cell death is a complex process where various upstream signaling pathways have been shown to either promote or prevent cell death (DeLegge et al., 2008). It has been reported that activation of AKT, a cell survival kinase, prevents dopaminergic neuronal cell death, while GSK3 β , an AKT upstream kinase, negatively regulates AKT and thereby promotes dopaminergic neuronal cell death (Nair et al., 2008). More specifically, we have shown that AKT phosphorylates MLK3 on a specific residue, Ser674, and down regulates MLK3 kinase activity and associated cell death (Figure 5A) (Barthwal et al., 2003; Mishra et al., 2007). Interestingly, ceramide, which attenuates AKT kinase activity (Mora et al., 2002), is also an activator of MLK3 and therefore an elevated level of ceramide, as seen in PD patients might downregulate the cell survival pathway by inhibiting AKT and concurrently activating the cell death pathway mediated via MLK3 or other MLK. This also could explain how ceramide might cause cell death in dopaminergic neurons.

Glycogen synthase kinase-3 β (GSK-3 β) has been identified primarily as a metabolic enzyme, regulating glycogen synthesis. However, GSK3 β has been implicated recently in a neuronal cell death pathway (Serenio et al., 2009) and reported to phosphorylate Tau-protein and promote tangle formation (Baum et al., 1996), a hallmark of Alzheimer disease. In a neuronal cell death model system, we have seen that nerve growth factor (NGF) deprivation causes neuronal cell death via activation of GSK3 β and MLK3 (Figure 5B) (Mishra et al., 2007). Further, investigation revealed that MLK3 was phosphorylated at two residues Ser789 and Ser793 by GSK3 β (Mishra et al., 2007). This phosphorylation of MLK3 by GSK3 β leads to its activation and concurrent activation of the MLK3-downstream kinase, JNK (Mishra et al., 2007). When, these two sites on MLK3 were mutated to non-phosphorable Ala, the activation of MLK3 by GSK3 β was blocked, and neuronal cell death upon NGF withdrawal also prevented (Mishra et al., 2007). It is reported that PD patients lack many growth factors (Mogi et al., 1999) and therefore it is worthy to envision that lack of growth factors in PD patients might lead to activation of a GSK3 β -MLK3-JNK pathway that ultimately can promote dopaminergic neuronal cell death. Our hypothesis was further confirmed recently when it was shown that GSK3 β inhibition indeed promotes the dopaminergic neuronal cell survival in an MPTP mouse model of PD (Wang et al., 2007).

In conclusion, our results suggest that MLK3 could serve as a primary MLK member that regulates dopaminergic neuronal cell death via JNK activation in response to neuro-inflammatory triggers like TNF- α . Upstream regulators of MLK3, AKT and GSK3 β , also might play central roles in regulating the cell death of dopaminergic neurons via MLK3 in PD patients. We believe that future investigation of MLK3 or GSK3 β may prove beneficial in halting dopaminergic neuronal loss during PD pathogenesis.

3.0 Summary

In this review, we describe the proapoptotic function of two kinases, PKC δ and MLK3, in neurotoxic models of Parkinson's disease (PD). Figures 6A and 6B summarize the overall pathway involving the caspase-3-dependent proteolytic activation of PKC δ and MLK3-JNK in PD models. Based on these results, PKC δ and MLK3 may become valid pharmacological targets for the development of a neuroprotective strategy against oxidative stress-induced

dopaminergic degeneration in PD. Further exploration of PKC δ and MLK3 signaling will also provide novel insights into the pathogenesis of PD.

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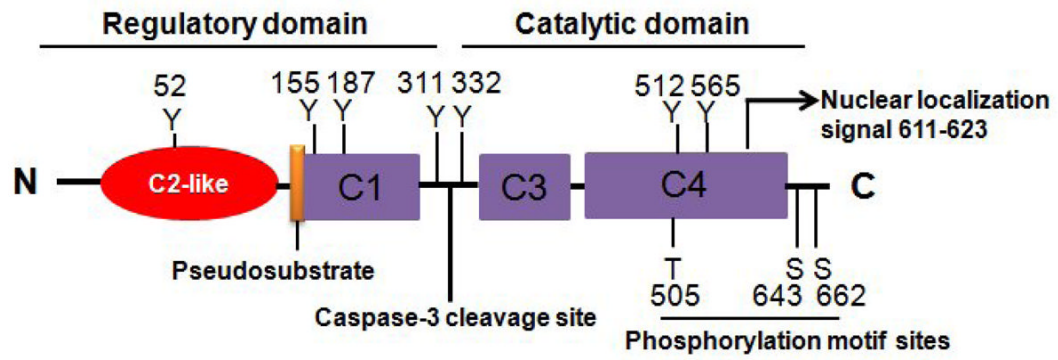


Figure 1. Schematic representation of the domain structure of PKC δ . Caspase-3 cleavage site, nuclear localization signal, and the phosphorylation sites including serine (S), threonine (T), and tyrosine (Y) residues are depicted.

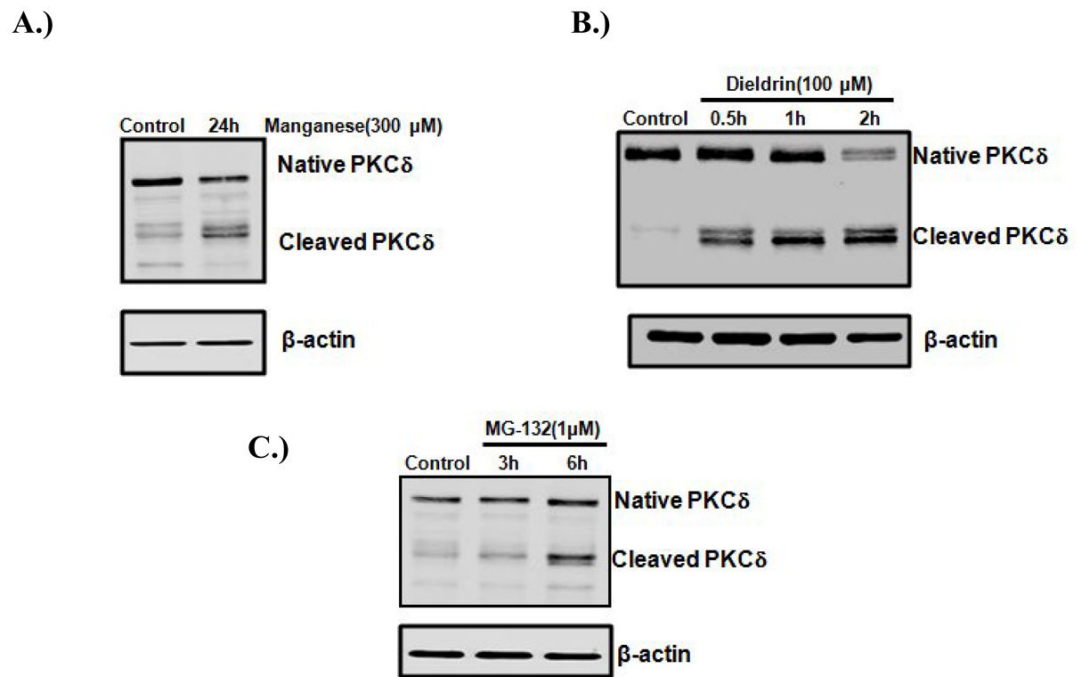


Figure 2. PKC δ cleavage in neurotoxicity cell culture models of PD. N27 dopaminergic neuronal cells were treated with environmental neurotoxicants manganese, dieldrin, or the classic proteasome inhibitor MG-132. After treatment, cells were collected and subjected to Western blot analysis of PKC δ . Native PKC δ (~74KDa) and cleaved fragments (~42KDa) are shown in each panel.

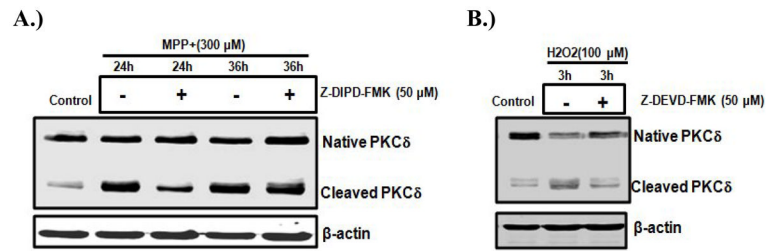
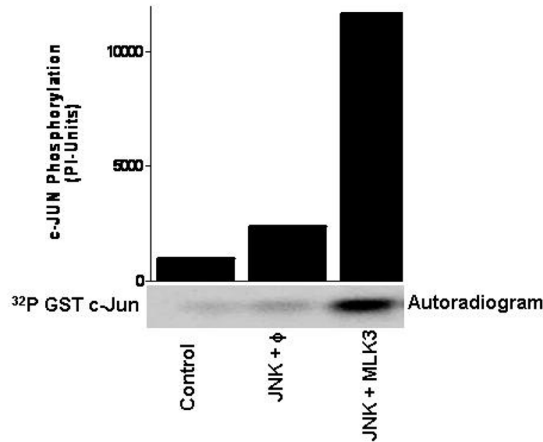


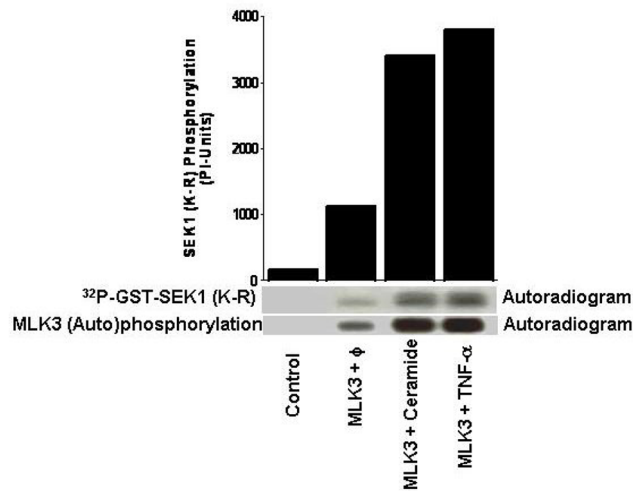
Figure 3.

Effect of caspase inhibitors on oxidative stress induced PKC δ cleavage in cell culture models of PD. N27 dopaminergic cells were treated with prooxidant H₂O₂ or the Parkinsonian toxicant MPP⁺ in the presence or absence of either 50 μ M Z-DIPD-FMK or 50 μ M z-DEVD-FMK. After treatment, cells were collected and subjected to Western blot analysis of PKC δ .

A.)

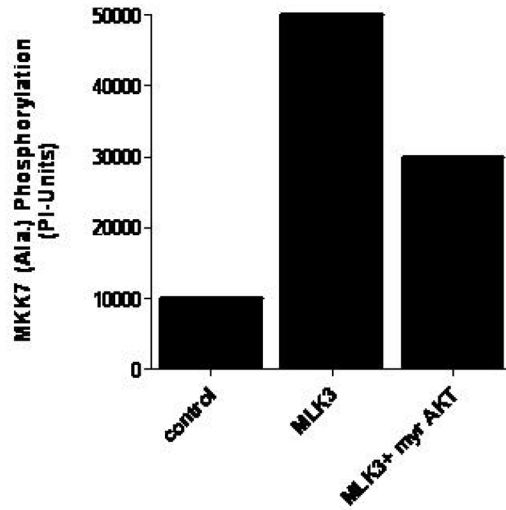


B.)

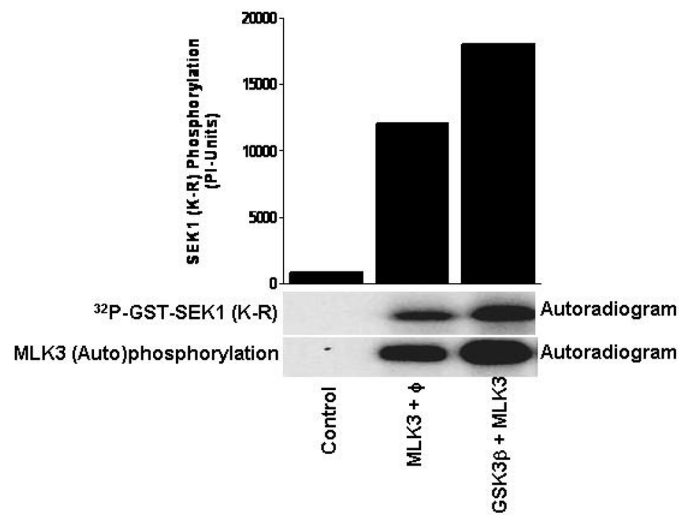
**Figure 4.**

MLK3 Activation: (A) MLK3 is a potent activator of JNK: Mammalian cells were transfected either with JNK alone or along with MLK3 expression vectors. The ectotopically expressed JNK was immunoprecipitated and kinase assay was performed using GST-Jun as the substrate. (B) MLK3 is activated by TNF α and ceramide: Mammalian cells were transfected with MLK3 expression plasmid and 36 hours post-transfection, cells were starved in 0.2% serum containing medium for 12 hours. The starved cells were either treated with ceramide (10 μ M) for 45 minutes or with TNF α (10 nM) for 30 minutes. MLK3 kinase activity was measured using GST-SEK1 protein as the substrate.

A.)



B.)

**Figure 5.**

Regulation of MLK3 activation by other kinases: (A) AKT negatively regulates MLK3 kinase activity: Mammalian cells were transfected with either MLK3 alone or along with active AKT (myr-AKT) expression vectors. The ectopically expressed MLK3 was immunoprecipitated and the kinase activity was measured using GST-MKK7 protein as the substrate (B) GSK3 β activates MLK3 kinase activity: The mammalian cells were transfected with either MLK3 alone or along with GSK3 β expression plasmids. The MLK3 kinase activity was measured as described in Figure 2.

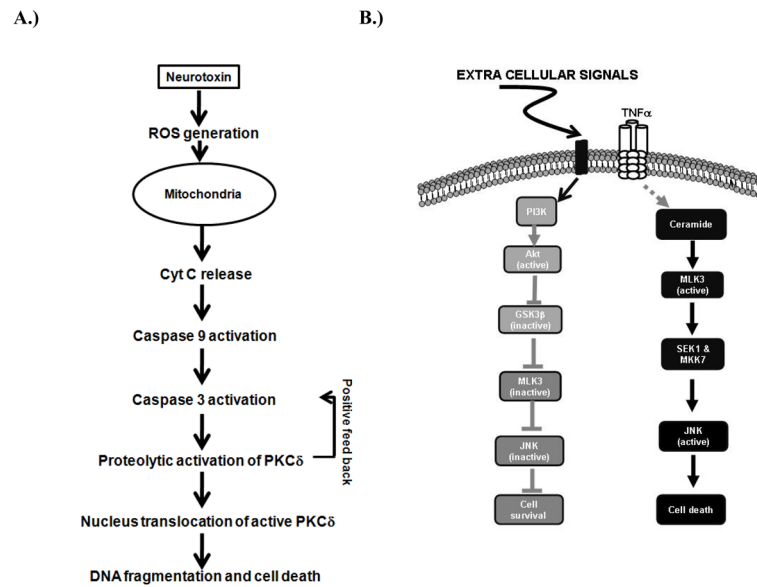


Figure 6.

(A) Schematic model showing the role of PKC δ in neurotoxin-induced neuronal apoptosis. Exposure to neurotoxins, such as MPP⁺, dieldrin, manganese and H₂O₂, induces early events of apoptosis including generation of reactive oxygen species (ROS), mitochondrial dysfunction, and release of cytochrome C into cytosol. The released cytosolic cytochrome C activates caspase-9, which subsequently activates caspase-3. Activated caspase-3 mediates proteolytic cleavage of PKC δ to produce an active PKC δ fragment. Proteolytic activation of PKC δ eventually contributes to cell death. The proteolytic activation of PKC δ can also regulate upstream the mitochondrial dependent caspase cascade by a positive feedback loop. (B). Schematic model for cell death and cell survival signaling pathway regulated by MLK3: Under growth factor sufficient conditions, PI3 kinase-AKT pathway remains active that leads to inhibition of GSK3 β . AKT also phosphorylates MLK3 at Ser674 site and attenuates MLK3 kinase activity. The inhibition of MLK3 kinase activity prevents the activation of JNK, finally leading to cell survival. On the contrary, the proinflammatory cytokines, TNF α activates MLK3 probably via ceramide generation or by some unknown mechanism. The activation of MLK3 by its agonists leads to activation of JNK and finally leading to cell death.