Programmed Cell Death in Parkinson’s Disease

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Parkinson’s disease is a debilitating disorder characterized by a progressive loss of dopaminergic neurons caused by programmed cell death. The aim of this review is to provide an up-to-date summary of the major programmed cell death pathways as they relate to PD. For a long time, programmed cell death has been synonymous with apoptosis but there now is evidence that other types of programmed cell death exist, such as autophagic cell death or programmed necrosis, and that these types of cell death are relevant to PD. The pathways and signals covered here include namely the death receptors, BCL-2 family, caspases, calpains, cdk5, p53, PARP-1, autophagy, mitophagy, mitochondrial fragmentation, and parthanatos. The review will present evidence from postmortem PD studies, toxin-induced models (especially MPTP/MPP+), 6-hydroxydopamine and rotenone, and from α-synuclein, LRRK2, Parkin, DJ-1, and PINK1 genetic models of PD, both in vitro and in vivo.

Cells have several mechanisms by which they can decide their own individual fate, in response to excessive damage, or unfavorable intracellular or extracellular conditions. This regulatory homeostatic function is genetically “programmed” and is therefore termed programmed cell death (PCD). Unlike classical necrosis, PCD is a process that requires ATP, changes in gene expression, and protein synthesis. This evolutionarily conserved biological phenomenon plays a vital role in many physiological processes, notably during development. On the flip side, PCD is a mechanism of cell demise in numerous pathologies, including progressive neurodegenerative disorders, such as Parkinson’s disease (PD).

Research into molecular pathways governing PCD has come a long way. Until Kerr first used the term apoptosis (Kerr et al. 1972), all cells were thought to die by necrosis. For a long time since then, the term PCD was synonymous with apoptosis. Today we know that apoptosis (or Type I cell death) is merely one type of PCD. Another type of PCD, autophagic cell death (or Type II cell death) has received a significant attention from PD researchers in the past few years. In addition, there is now sufficient evidence that necrosis, a process traditionally regarded as “passive,” may also be genetically programmed. The same stimulus can trigger multiple cell death pathways, depending on its degree and duration, brain region and cell type, and also depending on the bioenergetic state of the cell (Eguchi et al. 1997; Young et al. 2004). For example, MPP+ and other toxins may trigger apoptosis in lower doses, whereas higher

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doses cause necrosis (Elmore 2007). It is not rare to see more than one type of cell death coexist in the same PD tissue (Anglade et al. 1997; reviewed by Perier et al. 2011). In addition, there is an overlap in some biochemical and morphological features between the different types of cell death and therefore it is highly recommended to employ more than one assay to confirm the type of cell death (Elmore 2007).

The main goal of this work is to give a broad overview of what we currently know about PCD as it relates to PD. A number of cellular stressors play a role in PD, from defects in mitochondrial respiration and oxidative stress, to accumulation of protein aggregates. There are a number of cell death pathways that have been described. However, with a multitude of animal models of PD, the contribution of each pathway is very difficult to assess. To illustrate this, there has been some controversy on how much the classical apoptotic death contributes to dopaminergic death. Numerous experimental factors could contribute to such discrepancies, including timing of evaluation, concentration of a toxin, or levels of gene expression, animal strain, or cell type. The roles of some death-related pathways are controversial and some likely have dual roles, for example, autophagic processes have both prodeath and prosurvival functions depending on cellular context.

Unfortunately, to date, there is no effective disease-modifying treatment that would stop or slow down the progressive neuronal loss in PD. A number of neuroprotective compounds failed in clinical trials, a mixed lineage kinase inhibitor CEP-1347 that offered neuroprotection in a number of PD models is one such example. There are of course many possible reasons for this, one of them being that many of our animal models may not truly reflect the cellular pathology in PD. The identification of genetic causes of PD might help in this problem. However, genetic animal models have not been very helpful when it comes to the study of degenerative processes, because most do not show robust cell death—at least not in vertebrates.

Another big question is how does loss- or gain-of-function of these PD-related genes contribute to human PD. These genes play a role in some of the pathways described below and therefore their mutations may cause cell death in PD by disrupting physiological regulation of PCD (described below). However, whether these pathways are associated with PD processes in human PD is not clear.

Taken together, we can describe a multitude of death-related processes that go on in animal models of PD. However, the complexity of the types of death and how they relate to human PD, beyond the animal models of DA degeneration is not at all clear and probably represents an important hurdle to overcome in the next phases of PD research. Furthermore, understanding the interplay between the different pathways may be essential for designing an effective treatment.

**APOPTOSIS: TYPE I CELL DEATH**

Apoptosis is an evolutionarily well-conserved molecular process. It is also the most common and the best-described form of PCD. Although phenotypes of apoptotic cells may differ, typical morphological features include shrinking of the cell, membrane blebbing, compartmentalization, chromatin condensation, and DNA fragmentation. The advantage of this highly regulated process over classical necrosis is that during apoptosis the membrane integrity remains intact with the contents of the dying cell enclosed within apoptotic bodies, without releasing them into an extracellular space. The dying cell expresses cell surface markers that target it for recognition and phagocytosis by adjacent cells, without activating an inflammatory response. Therefore, this type of cell death may be regarded as “cleaner” and “preferred,” provided the cell has enough energy to go through with the death program.

Numerous assays have been developed to detect apoptosis, including ultrastructural analysis assessing cellular morphology, testing for DNA fragmentation, detection of caspases, their cleaved substrates, or regulators of apoptosis, or methods assessing mitochondrial function (comprehensively reviewed by Elmore 2007).

The first indication that apoptosis may be involved in neuronal death in PD came from in...
in vitro studies with neurotoxin MPP+ (Dipasquale et al. 1991). For some time however, the question of whether or not neurons die by apoptosis in PD had been a somewhat controversial issue. Although several laboratories found apoptotic neurons in postmortem substantia nigra (SNc) of PD patients (Mochizuki et al. 1996; Anglade et al. 1997; Tompkins et al. 1997), these results could not be replicated by others (Kosel et al. 1997; Banati et al. 1998; Wullner et al. 1999). This was likely in part because of the fact that only a very small number of neurons undergo apoptosis at any given time (apoptosis is a quick process—from initiation to completion of apoptosis takes only 2–3 h (Elmore 2007), partly because of methodological limitations of the experimental technique used to detect apoptotic neurons by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining to detect DNA fragmentation in situ (Tatton 2000; Tatton et al. 2003), and partly because of very narrow morphological criteria by which apoptosis was defined at the time (Tatton et al. 2003). Later, the role of apoptosis in PD pathology was confirmed in human tissue postmortem by TUNEL staining with simultaneous application of fluorescent DNA binding dyes to detect chromatin condensation and DNA cleavage in the same nucleus (Tatton et al. 1998; Tatton 2000) and increased immunostaining for caspase-3, caspase-8, and caspase-9 in nigrostriatal dopaminergic neurons (Hartmann et al. 2000, 2001b; Tatton 2000) and elevated activities of caspase 1 and caspase 3 in SNc (Mogi et al. 2000).

There are a number of animal models of PD (Dauer and Przedborski 2003). MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a commonly used agent in PD research. It is a precursor of neurotoxin 1-methyl-4-phenylpyridinium (MPP+) that inhibits complex I of the mitochondrial electron transport chain (Nicklas et al. 1987), causing oxidative stress and energy failure. MPP+ treated cells are a common in vitro model of PD cell death, and MPTP-injected mouse is used to model PD in vivo. Similar to postmortem studies, increased numbers of apoptotic neurons and apoptotic markers were also observed in dopaminergic neurons in SNc of MPTP-treated mice (Tatton and Kish 1997; Viswanath et al. 2001), although an acute administration of high doses of MPTP, unlike the subacute regimen, causes predominantly a nonapoptotic cell death (Jackson-Lewis et al. 1995; Przedborski and Vila 2003). Similarly, MPP+ or rotenone cause apoptosis in low doses, but necrosis accompanied by gliosis in higher concentrations (Hartley et al. 1994). Other complex I inhibitors, such as paraquat or rotenone, are also used to model PD.

Another in vivo model of PD is the 6-hydroxydopamine (6-OHDA) rat model, in which dopaminergic neurons are lesioned by injecting neurotoxin 6-OHDA into the medial forebrain bundle, SNc, or striatum. Nigrostriatal neurons of these rats are also TUNEL positive and display a typical morphology of cells undergoing apoptosis (Zuch et al. 2000), although this was not seen in all studies (Jeon et al. 1995). Similarly, treating PC12 or primary microglial cells with this neurotoxin also caused apoptosis (Takai et al. 1998).

Furthermore, products of PD genes are linked to PCD. Overexpression of wild type or mutant α-synuclein in cultured neurons causes apoptosis (Saha et al. 2000) and increases sensitivity to apoptotic cell death (Tanaka et al. 2001), although α-synuclein plays a dual role depending on its expression levels (Seo et al. 2002). A53T mutant α-synuclein mice have markers of apoptosis in neocortex, brainstem, and spinal cord (Martin et al. 2006; Smith et al. 2010), and conversely down-regulation of α-synuclein protected from MPTP cell death in vivo (Hayashita-Kinoh et al. 2006). In contrast, some investigators show that overexpression of wild type α-synuclein protects from apoptosis (Alves Da Costa et al. 2002; Li and Lee 2005).

Overexpressing pathogenic mutant LRRK2 caused apoptosis in primary neurons (MacLeod et al. 2006), in neuroblastoma cells (Iaccarino et al. 2007; Kanao et al. 2010), or in Drosophila (Kanao et al. 2010). Knocking-out LRRK2 increased number of TUNEL positive neurons and activated caspase-3 in mouse kidneys (Tong et al. 2010).

Wild type (but not mutant) DJ-1 protects from apoptosis (Canet-Aviles et al. 2004; Junn...
et al. 2005; Xu et al. 2005b), specifically by inhibiting p53 transcriptional activity (Fan et al. 2008). DJ-1 deficiency increased oxidative stress-induced apoptotic cell death (Martinat et al. 2004; Kim et al. 2005). Similarly, pathogenic mutant or DJ-1 knockdown cells were hypersensitive to apoptosis (Yokota et al. 2003; Shinbo et al. 2006). These results are supported by data in Drosophila (Yang et al. 2005).

Parkin prevents cytochrome c release in an E3 ubiquitin ligase-dependent manner (Darios et al. 2003; Berger et al. 2009) and protects from apoptosis in dopaminergic neurons (Staropoli et al. 2003; Jiang et al. 2004), in 6-OHDA-treated rat (Vercammen et al. 2006), and in Drosophila by suppressing JNK signaling (Hwang et al. 2010). Overexpressing Parkin protects SH-SY5Y cells from 6-OHDA toxicity, whereas genetic ablation of Parkin increases susceptibility of neurons to rotenone (Casarejos et al. 2006) and can even cause apoptosis in the absence of a neurotoxin (Machida et al. 2005). Flight muscles of Drosophila Parkin null mutants undergo apoptotic degeneration (Greene et al. 2003) and have increased sensitivity to oxidative stress (Pesah et al. 2004).

Overexpression of wild-type PINK1 has a prosurvival effect (Valente et al. 2004; Petit et al. 2005; Pridgeon et al. 2007) by inhibiting opening of the mitochondrial PTP (Wang et al. 2007). However, mutant PINK1 (Klinkenberg et al. 2010) or depletion of PINK1 (Deng et al. 2005; Gandhi et al. 2009; Mei et al. 2009; Wang et al. 2011a) sensitizes to apoptotic death.

Caspases: The Key Molecular Players in Apoptosis

Caspases are a family of cysteine proteases that inactivate prosurvival proteins and activate proapoptotic proteins in an amplifying cascade. They cleave specific sites at aspartate residues of target substrates. Activation of caspases is often used as a marker of apoptosis.

There are two types of caspases: the ones involved in the initiation phase of apoptotic cell death called initiator caspases (most importantly, caspase-8 and caspase-9), and the ones involved in its execution called executioner caspases (caspase-3, -6, and -7). The initiator caspases cleave and activate executioner caspases. The executioner caspases are then responsible for cleaving numerous substrates, which ultimately leads to cell death. At least hundreds of other substrates of caspase-3 have been identified.

Caspase activation, as one would expect, is a highly regulated process. Initiator caspases are expressed as inactive proenzymes. Originally it was thought that initiator caspases become activated by a proteolytical cleavage of their prodomain. However, new evidence shows that cleavage of initiator caspases is neither required, nor sufficient for their activation (Fuentes-Prior and Salvesen 2004), and that they become activated by dimerization. This is important to keep in mind when evaluating published literature. Executioner caspases are already present in the cell as dimers and become activated by cleavage that leads to intramolecular rearrangements (Tait and Green 2010). Caspases can be inhibited by some members of a family called “inhibitors of apoptosis” (IAPs), most notably by XIAP. XIAP binds and thus prevents activation of caspase-3, caspase-7, and caspase-9. Thus, overexpressing XIAP protects from cell death in MPTP models of PD (Crocker et al. 2003a).

Two Roads to Apoptotic Death

So how do caspases become activated? Multiple cellular events and/or external stimuli may trigger an apoptotic program. There are two major apoptotic pathways, extrinsic and intrinsic. Although each pathway employs a different set of initiator caspases, both share the same executioner caspases.

In the case of the extrinsic (death receptor) pathway, apoptosis is triggered by activation of specialized trimeric cell surface receptors termed “death receptors.” These receptors are members of the TNFR family and include a tumor necrosis factor (TNF) receptor 1 (TNFR1), Fas (sometimes called CD95 or APO-1), DR3, DR6, and TRAIL receptors. Their ligands are members of the TNF superfamily and are referred to as “death ligands.” These include...
TNF, CD95 ligand (sometimes called Fas ligand, Fasl), and TRAIL. These transmembrane receptors contain an intracellular death domain (death fold) and essentially act as cell sensors. Briefly, on their activation by the trimeric ligand, the receptors change conformation and their intracellular domain associates with a cytoplasmic adaptor protein that contains death effector domain TRADD (when the ligand is TNF, or FADD when the ligand is Fasl), RIP1, cellular inhibitor of apoptosis (cIAP1), cIAP2, TRAF2, and TRAF5. FADD then binds procaspase 8 (or procaspase 10), forming a death-inducing signaling complex (DISC). As a result, caspase 8/10 gets activated, which triggers apoptosis. DISC signaling complex can dissociate from the receptor and form signaling complex II that also contributes to caspase-8 activation (Oberst and Green 2011; Lavrik and Krammer 2012). Alternatively, Fas can interact with Daxx, which activates apoptosis-signaling kinase ASK1 (Yang et al. 1997b). ASK1 then activates JNK (Ichijo et al. 1997). This alternate pathway is relevant, for example, in DJ-1 neurobiology (Junn et al. 2005) and paraquat (Yang et al. 2009) or MPTP toxicity (Lee et al. 2012).

Despite some evidence supporting the role of the death receptor pathway in PD cell death (Boka et al. 1994; de la Monte et al. 1998; Mogi et al. 2000; Hayley et al. 2004; Ho et al. 2009; Simunovic et al. 2009; Fu et al. 2011), the mitochondrial pathway may play a major role in triggering apoptosis in PD.

The intrinsic (mitochondrial) pathway is activated by most of the known cellular stressors—like DNA damage, reactive oxygen species (ROS), or loss of trophic support. The central phenomenon in this pathway is the release of cytochrome c (together with other soluble molecules) from mitochondrial intermembrane space into the cytosol. This is the outcome of an irreversible and rapidly progressing process called mitochondrial outer membrane permeabilization (MOMP). MOMP can either occur by the pore-forming activities of pro-apoptotic BCL-2 family members (especially BAX and BAK), or thanks to opening of a specific multiprotein channel called the permeability transition pore (PTP) that intersects through the outer and inner mitochondrial membrane. This leads to the mitochondrial permeability transition (MPT, also known as mitochondrial depolarization) characterized by dissipation of the inner mitochondrial membrane potential, diminished ATP synthesis, ROS production, mitochondrial swelling, and rupture.

**BCL-2: A Family Divided**

The integrity of mitochondrial membrane has to be tightly regulated. This function is served by the BCL-2 family of proteins (Yang et al. 1997a). Based on their role in regulation of apoptosis, the members can be divided into three distinct subfamilies.

First, there are the proapoptotic BCL-2 proteins (BAX, BAK, BOK) that cause MOMP. For example, BAX, in its inactive state, is found ubiquitously in the cytosol. Activation of BAX requires induction of its transcription and translocation. Thus, in response to a death stimulus, transcription factor p53 induces transcription of BAX, which then undergoes mitochondrial translocation. This translocation requires JNK-dependent activation of the BH3-only protein BIM (Tournier et al. 2000; Putcha et al. 2001; Whitfield et al. 2001; Lei and Davis 2003); JNK can also directly activate BAD (Donovan et al. 2002). JNK is activated after MPTP administration, and blocking JNK is neuroprotective in this cell death paradigm (Saporito et al. 1999; Xia et al. 2001), or in 6-OHDA-induced neuronal death (Ries et al. 2008). Parkin also exerts its antiapoptotic effects partly by suppressing JNK (Cha et al. 2005).

Once inserted into the mitochondrial membrane, BAX oligomerizes and forms holes in it, in collaboration with BID and mitochondrial BAK (Dewson et al. 2009). BAX activation has repeatedly been reported in MPTP mice, 6-OHDA-lesioned rats, and in brain tissue of PD patients (Hassouna et al. 1996; Tatton 2000; Hartmann et al. 2001a; Vila et al. 2001; Perier et al. 2005), or when treating PC12 cells with 6-OHDA in vitro (Blum et al. 1997) or overexpressing high levels of α-synuclein (Seo et al. 2002)—although not all investigators observed this effect (Jellinger 2000). Conversely, BAX...
suppression is protective. Thus, BAX or BAK deficiency protected against MPTP or paraquat toxicity in vivo (Vila et al. 2001; Fei et al. 2008), and targeting either BAX transcriptional induction or its mitochondrial translocation significantly protected dopaminergic neurons from MPTP toxicity (Perier et al. 2007). Moreover, DJ-1 overexpression decreases BAX expression (Fan et al. 2008).

The second subfamily comprises prosurvival BCL-2 proteins (for example, BCL-2, BCL-xL, BCL-W, MCL-1, and others) that prevent MOMP. Specifically, BCL-2 prevents oligomerization of BAX (Dlugosz et al. 2006). Therefore, BCL-2 overexpression protects several types of neurons from apoptosis, whereas knocking BCL-2 out makes them more susceptible to apoptosis. In line with these findings, MPTP treatment decreased expression of BCL-2 in mouse SNc (Vila et al. 2001), BCL-2 overexpression protected from MPTP/MPP+ toxicity (Offen et al. 1998; Yang et al. 1998; Vila et al. 2001), and 6-OHDA toxicity in vivo and in vitro (Offen et al. 1998; Takai et al. 1998; Yamada et al. 1999; Natsume et al. 2001). Interestingly, some (Mogi et al. 1996; Marshall et al. 1997; Hartmann et al. 2002) although not all (Vyas et al. 1997; Jellinger 2000) investigators observed an increased expression of BCL-2 or BCL-xL in SNc or mesencephalon of PD patients. This might suggest a compensatory mechanism. High cellular concentration of α-synuclein also down-regulates BCL-2 and BCL-xL (Seo et al. 2002).

The third subfamily, BH-3-only, promotes apoptosis indirectly—by sequestering the antiapoptotic BCL-2 proteins. The BH3-only elements include BID, BAD, BIM, BLK, PUMA, NOXA, and BNIP3L. BAD can heterodimerize with BCL-xL or BCL-2, which neutralizes their protective effect and promotes cell death (Yang et al. 1995). BID, BIM, and PUMA can trigger oligomerization of BAX or BAK and participate in MOMP. Expression of BIM was markedly increased in response to MPP+ treatment, and BIM knockdown is protected from cell death in this paradigm (Liou et al. 2005). LRRK2 induces expression of BIM and HID by phosphorylating transcription factor FoxO (Kanao et al. 2010). siRNA-mediated knockdown of BNIP3 or NOXA offered neuroprotection from paraquat (Fei et al. 2008).

In essence, the cellular fate is decided based on the ratio and interactions between these pro- and antiapoptotic regulators (Green and Reed 1998). For a review about the BCL-2 family, please see Shacka and Roth (2005).

Time for Caspase Activation

Once the mitochondrial membrane is permeabilized in response to an apoptotic stimulus, a number of molecules can freely diffuse from the mitochondria, including cytochrome c and procaspase-9. Cytochrome c induces polymerization of cytosolic Apaf-1, which is the adaptor protein of the mitochondrial pathway and is required for activation of caspase-9. Cytochrome c–Apaf-1 complex then binds procaspase-9 via a homologous death fold called CARD domain. In the next step, caspase-9 becomes activated and Apaf-1 and caspase-9 form the apoptosome complex. This apoptosome complex then plays a role in activation of caspase-3. Cells also produce heat shock proteins (Hsp) in response to stress, for example, Hsp90 and Hsp70. These directly associate with Apaf-1 and prevent caspases from being recruited to the apoptosome complex. Hsp70 can also bind AIF and block its proapoptotic activity (Ravagnan et al. 2001).

As noted earlier, IAPs can inhibit apoptosis by preventing activation of caspases. To add an extra layer of regulation, two other mitochondrial proteins that are also released from the intermembrane mitochondrial space can bind and inactivate IAPs. These are called Smac/DIABLO (Green and Reed 1998; Du et al. 2000; Verhagen et al. 2000), and Omi/HtrA2. For example, Smac/DIABLO is released following an insult by 6-OHDA (Gorman et al. 2005), although its role in apoptosis is not clear—Smac/DIABLO knockout mice have no obvious phenotype and their cells do undergo apoptosis after a variety of stimuli (Okada et al. 2002). Please see reviews by Ekshyyan and Aw (2004) and Yakovlev and Faden (2004).
ER Response

In addition to mitochondria, endoplasmic reticulum (ER) also plays a role in apoptosis. ER is an essential organelle for synthesis, proper folding and posttranslational modifications of proteins, and for maintaining calcium homeostasis. Excessive amount of cytosolic calcium can affect PCD by inducing MPT, by affecting the BCL-2 family proteins, by activating mitochondrial fission protein Drp1 (Xu et al. 2005a), or by persistently activating calpains (Harwood et al. 2005; Vosler et al. 2008). There is an increased calpain expression (Mouatt-Prigent et al. 1996) or activity in the mesencephalon of PD patients (Crocker et al. 2003b), 6-OHDA-treated rats (Grant et al. 2009), MPTP mice (Crocker et al. 2003b), or rotenone-treated animals (Chen et al. 2006). Pharmacological or genetic inhibition of calpains significantly protected dopaminergic neurons from death in MPTP mice (Crocker et al. 2003b).

Disturbances in ER function (aberrant calcium homeostasis, or dysfunction in the ubiquitin proteasome system and accumulation of misfolded proteins), initiates an evolutionarily conserved cell stress response: the unfolded protein response (UPR). During UPR, cell suppresses global protein synthesis while inducing expression of specific proteins, for example, transcription factor CHOP/Gadd153, or ER chaperone proteins like glucose-regulated protein 78 (Bip/Grp78). However, if ER dysfunction is severe or prolonged, UPR can trigger apoptosis, although the mechanism is not fully understood (reviewed by Burke 2008). Caspase-12 (its closest human homolog is caspase-4) localizes to the ER and seems to be especially important in this pathway. The proapoptotic members of the BCL-2 family, such as BAX and BAK, are also ER expressed (Scorrano et al. 2003; Zong et al. 2003) and are required for ER stress-induced apoptosis, together with PUMA (Wei et al. 2001; Zong et al. 2001; Ruiz-Vela et al. 2005). The pathway is regulated by the MAPK pathway (Nishitoh et al. 2002; Lei and Davis 2003; Putcha et al. 2003).

Dysfunctional UPS and protein misfolding are some of the key pathological features of PD and activation of UPR seems to be an early event in PD (Hoozemans et al. 2012). Parkin mutations are associated with ER stress-induced cell death, while overexpressing wild-type Parkin was protective (Imai et al. 2000; Dawson and Dawson 2003). Parkin is transcriptionally regulated by ER stress (Imai et al. 2000), specifically by ATF4 (Bouman et al. 2011). α-Synuclein also activates the UPR pathway (Sugeno et al. 2008; Bellucci et al. 2011). In in vitro models of PD (6-OHDA treated neuroblastoma cells) or in in vivo models of PD (MPTP mouse or rabbit, 6-OHDA rat) there is a markedly increased expression of CHOP/Gadd153 and/or Bip/Grp78 (Ghribi et al. 2003; Silva et al. 2005; Yamanur et al. 2006). Moreover, stress-induced transcription factors such as ATF3, ATF4, and/or CHOP were up-regulated in 6-OHDA treated cells (Chen et al. 2004; Holtz et al. 2005). Therefore, the reticular pathway is highly relevant for PD pathology.

The Cdk5 Link

One of the pathways regulated by calpains is the cyclin-dependent kinase 5 (Cdk5) pathway (Smith et al. 2006). Ample evidence suggests that Cdk5 plays a key role in PD cell death. Unlike other Cdk family members, this serine/threonine kinase does not seem to be critically involved in cell cycle. Cdk5 is preferentially expressed in neurons and its activity is regulated by p35/p39. It is not however entirely clear how Cdk5 fits into the apoptotic pathway.

There is evidence of p35 and/or Cdk5 immunoreactivity in Lewy bodies in brains from PD patients (Brion and Couck 1995; Nakamura et al. 1997). Also in MPTP mouse, Cdk5 expression is elevated. In this model, Cdk5 associates with a calpain-cleaved form of p35, p25, becomes overactive and translocates to the nucleus (Smith et al. 2003, 2006). Increased Cdk5/p25 expression has also been shown in brains of PD patients. Once in the nucleus, phosphorylation by Cdk5 signals for death by reducing activity of a prosurvival transcription factor MEF2D (Smith et al. 2006) and of a DNA repair enzyme Ape1 (Huang et al. 2010). In addition, Cdk5 exacerbates oxidative stress by phosphorylating...
and thus inhibiting the peroxidase activity of an antioxidant enzyme Prx2 (Qu et al. 2007). Moreover, Cdk5 phosphorylation of Parkin regulates its ubiquitin-ligase activity and protein aggregation (Avraham et al. 2007). α-Synuclein is another one of many other Cdk5 substrates (see reviews by Camins et al. 2006; Cheung and Ip 2011; Lopes and Agostinho 2011).

A nonspecific pharmacological inhibition of Cdk5 (Smith et al. 2003), or genetic inhibition of Cdk5 or p35 attenuated neuronal loss induced by MPP+ and MPTP in vitro and in vivo, respectively (Smith et al. 2006; Qu et al. 2007). However, inhibiting Cdk5 was also protective in necrotic death paradigms (Weishaupt et al. 2003).

Dysregulation of Cdk5 has also been associated with an attempted cell cycle re-entry. Because neurons are regarded as terminally differentiated postmitotic cells, the cell cycle is never completed and these neurons activate proapoptotic proteins of the BCL-2 family and caspase-3, leading to apoptosis (Konishi et al. 2002). This topic is reviewed by Krantic et al. (2005).

p53 and DNA Damage Pathway

The cells have several mechanisms to protect their DNA from genotoxic stress, for example, by p53-mediated activation of DNA damage proteins. However, if the damage is beyond repair p53 will initiate apoptosis. p53 acts as a transcriptional regulator: it can increase transcription of BAX, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Xiang et al. 1998; Chen et al. 1999; Fan et al. 2008), NOXA and PUMA (Oda et al. 2000; Jeffers et al. 2003; Villunger et al. 2003), while repressing antiapoptotic IAP genes (Hoffman et al. 2002). However, p53 can also, on its mitochondrial translocation, induce oligomerization of Bax and Bak and sterically block the antiapoptotic BCL-2 and BCL-xL (Wolff et al. 2008; reviewed by Speidel 2010). Furthermore, there is an alternative cytosolic p53 death pathway that can directly activate cytosolic Bax (Chipuk et al. 2005). Therefore, p53 is one of the key effectors regulating apoptosis.

p53 immunoreactivity is increased in brains of PD patients (de la Monte et al. 1998; da Costa et al. 2009) and it is accompanied by an increased phosphorylation of p38 MAPK that phosphorylates and stabilizes p53 (Karunakaran et al. 2008). p38 inhibition protected primary dopaminergic neurons from MPP+ induced cell death (Karunakaran et al. 2008). p53 is also activated by 6-OHDA (Blum et al. 1997; Liang et al. 2007) and MPTP (Mandir et al. 2002). In vitro, MPP+, 6-OHDA, or rotenone also increase levels or activity of p53 (Bruchelt et al. 1991; Kitamura et al. 1998; Xu et al. 2005c; Chen et al. 2006; Nair 2006; Gomez-Lazaro et al. 2008), although p53 may not always be the mediator of cell death in these paradigms (Gomez-Lazaro et al. 2008). Similarly, A53T transgenic mice have an increased expression of p53 (Martin et al. 2006), whereas overexpression of wild-type α-synuclein in cultured neurons diminishes basal levels and transcriptional activity of p53 (Alves Da Costa et al. 2002). Wild-type (but not mutant) Parkin binds p53 promoter and acts as a p53 transcriptional repressor (da Costa et al. 2009). DJ-1 also represses p53 (Breaut et al. 2007; Fan et al. 2008). Finally, pharmacological inhibition (Duan et al. 2002) or genetic ablation (Timmer et al. 1996) attenuates MPTP-induced neuronal loss. Therefore, p53 seems to be an important regulator of PCD in PD.

Mitochondrial Fragmentation

Mitochondria are highly dynamic organelles that constantly undergo fission and fusion. Their dynamic nature allows them to better respond to intracellular conditions. By fusion with neighboring mitochondria, the damaged organelle can restore its function and protect mtDNA (Nakada et al. 2001; Ono et al. 2001). The ability of mitochondria to fuse is also required for optimal ATP production (Chen 2006). Fission on the other hand is important for removal of damaged mitochondria through a process termed mitophagy (reviewed by Suen et al. 2008). In addition, disrupting balance between fission and fusion can negatively impact mitochondrial transport and distribution (Li et al. 2004; Chen et al. 2007; Sheng and Cai 2012).
Before a link between autophagy and mitochondrial dynamics has been established (Twig et al. 2008; discussed elsewhere), apoptotic death was known to be accompanied by mitochondrial fragmentation (reviewed by Bossy-Wetzel et al. 2003; Suen et al. 2008). For example, MPP\textsuperscript{+} or rotenone causes fragmentation in sublethal concentrations (Barsoum et al. 2006). Although some suggest that this fragmentation is caused by a decreased fusion (Guo et al. 2007; Shen et al. 2007), most evidence points toward an excessive fission: in response to an apoptotic stimulus, Drp1 (a key molecule in fission) translocates from cytoplasm to the mitochondrial membrane in which it colocalizes with proapoptotic BAX and BAK and causes fission (Karbowski et al. 2002). Indeed, caspase activation and cell death can be delayed by blocking mitochondrial fission (Frank et al. 2001). In contrast, during resting state, BAX and BAK bind and activate profusion molecule Mfn2 (Karbowski et al. 2006; Brooks et al. 2007). Cdk5 is another molecule that regulates mitochondrial fission (Meuer et al. 2007). This is a rapidly growing area of research that is, however, beyond the scope of this work.

**AUTOPHAGIC CELL DEATH (TYPE II CELL DEATH)**

Autophagy is a cellular process that is currently a subject of an intense research in our field. This work will only provide a brief overview of autophagic cell death; for additional material on autophagy see Lynch-Day et al. (2012).

In essence, autophagy is a recycling process by which a cell can dispose of damaged organelles and long-lived proteins by sequestering them in a bilayer autophagic vacuole called autophagosome and transporting them to lysosome for enzymatic degradation. The cell can then reuse these components and resultant energy to survive under stress conditions. Therefore, starvation-induced autophagy is protective (Lum et al. 2005) and blocking it would trigger apoptosis (Boya et al. 2005; Gonzalez-Polo et al. 2005).

However, autophagic processes may also represent an alternative and considerably less-well understood pathway to PCD. This pathway is not dependent on caspases, and cells undergoing autophagic cell death are characterized by presence of large cytoplasmic vacuoles and expression of molecular markers of autophagy. Some paradigms for triggering autophagic death include activation of autophagic machinery while blocking lysosomal fusion/ degradation, or while blocking apoptosis (by a non-specific pan-caspase inhibitor Z-VAD-FMK), by BCL-2 or BCL-xL overexpression, or by knocking out BAX and BAK (Shimizu et al. 2004). Although autophagic and apoptotic death are two distinct death pathways, they share some molecular components (Pattingre et al. 2005; Maiuri et al. 2007a). For example, a proteolytically activated autophagy protein Atg5 can play a proapoptotic role on translocation to mitochondria and triggering MOMP (Yousefi et al. 2006). Please see the following review on this cross-talk (Maiuri et al. 2007b).

However, there are two important points to be made. First, activation of autophagy in a dying cell does not automatically mean autophagy is the cause of death—it may also suggest that the cell has activated autophagy as the last resort effort to prevent cell death, or perhaps that autophagy is employed as a mean of obtaining energy to trigger apoptosis (reviewed by Maiuri et al. 2007b). Therefore, one can find evidence of apoptosis and autophagy within the same cell—but this type of cell death would not be classified as autophagic death (Green 2011).

Second, an increased autophagic vacuolization may not always mean that autophagy is increased—it may well be that there is a problem with fusion of autophagosomes with lysosomes and/or with elimination of unwanted cellular components. Therefore, it is important to measure "autophagic flux" defined as the flow of autophagosomes from their formation to their fusion with lysosomes. This can be done by inhibiting fusion with bafilomycin A that blocks lysosomal hydrolase and autophagosome degradation.

Substantial evidence implicates autophagy in neurodegeneration. For example, knocking out Atg5 or Atg7 causes apoptotic death accompanied by accumulation of cytoplasmic inclusion...
bodies and protein aggregates (Hara et al. 2006; Komatsu et al. 2006). There is also evidence of autophagy alongside of apoptosis in PD patients (Anglade et al. 1997). Furthermore, PD genes LRRK2 (Plowey et al. 2008; Alegre-Abarrategui et al. 2009; Alegre-Abarrategui and Wade-Martins 2009; Tong et al. 2010; Hakimi et al. 2011; Ramonet et al. 2011; Gomez-Suaga et al. 2012), DJ-1 (Gonzalez-Polo et al. 2009; Irrcher et al. 2010; Krebiehl et al. 2010; Ren et al. 2010; McCoy and Cookson 2011), and α-synuclein (Winslow and Rubinsztein 2011) are all implicated in regulation of autophagy. The role of PINK1 and Parkin in mitophagy is described below. Increased formation of autophagosomes is also seen—MPP⁺, 6-OHDA, rotenone, or paraquat toxicity (Zhu et al. 2007; Dagda et al. 2008; Dadakhujaev et al. 2010; Niso-Santano et al. 2011). Other autophagy players involved in PD cell death include Cdk5 (Wong et al. 2011), p53 (Feng et al. 2005; Crighton et al. 2006; Tasdemir et al. 2008), or ER stress (Pandey et al. 2007).

Unfortunately, no specific pharmacological inhibitors of autophagy are currently available (Chu 2011). Knocking-down key autophagy players (Atg5, Atg7, Atg8) by siRNA protected primary neurons from MPP⁺ induced cell death (Chu et al. 2007). Similarly, suppressing autophagy indirectly by activating key autophagy regulator, mTOR, protected from oxidative stress in dopaminergic neurons, whereas mTOR inhibitor rapamycin caused exacerbation (Choi et al. 2010). In contrast, rapamycin partially protected from parauat toxicity in Drosophila (Ravikumar et al. 2006), and showed neuroprotection in other neurodegeneration models (Ravikumar et al. 2004). Therefore, the outcome of autophagy regulation may be context-dependent and we certainly need specific pharmacological inhibitors of autophagy.

**Mitophagy**

Cells can employ autophagy to remove damaged organelles, such as mitochondria. Mounting evidence suggests dysfunctional mitochondrial autophagy (mitophagy) may be part of the cellular pathology in PD. Mitophagy allows removal of damaged mitochondria (Dagda et al. 2009), unless the stimulus reaches threshold for triggering apoptosis (Rodriguez-Enriquez et al. 2006). For example, treating primary cortical neurons or neuroblastoma cells with MPP⁺ induces MOMP, mitophagy, and autophagic cell death (Zhu et al. 2007). Although mitochondrial fragmentation precedes mitophagy, it is not sufficient on its own to trigger it.

PD genes have been implicated in mitophagy regulation. Loss of DJ-I (Thomas et al. 2011) or overexpression of mutant α-synuclein (Choubey et al. 2011) activates mitophagy. It is, however, Parkin and PINK1 that seem to be the essential components of the mitochondrial quality control pathway, although some aspects of the pathway still need to be clarified. The initial discovery was made in Drosophila in which PINK1 loss-of-function flies have a profound phenotype that includes loss of dopaminergic neurons, swollen and damaged mitochondria, and wing posture deficits (Park et al. 2006). This phenotype was strikingly similar to Parkin loss-of-function flies (Greene et al. 2003; Cha et al. 2005). Remarkably Parkin is able to rescue the PINK1 loss-of-function phenotype, whereas PINK1 overexpression has no major effect in Parkin mutants (Park et al. 2006). Similar results were reported by Yang et al. (2006). This, for the first time, suggested that PINK1 and Parkin are part of the same pathway, PINK1 being upstream of Parkin. Briefly, under normal conditions, mitochondrial PARK1 gets rapidly degraded. However, during loss of mitochondrial membrane potential, PINK1 processing is blocked and full-length PINK1 becomes stabilized on the outer mitochondrial membrane (Lin and Kang 2008; Matsuda et al. 2010; Narendra et al. 2010). This serves as a signal that attracts Parkin to the damaged mitochondrion. Parkin then poly-ubiquitinates proteins at the mitochondrial membrane, including Mfn1 and Mfn2 (Gegg et al. 2010; Ziviani et al. 2010), which targets them for degradation by the UPS. This promotes fission (by preventing fusion) (Tanaka et al. 2010), and recruits p62 that targets the damaged mitochondrial for degradation by Atg5-dependent mitophagy, although the requirement of
p62 for mitophagy is presently controversial (Narendra et al. 2008, 2010; Vives-Bauza et al. 2010). Recent data show that this pathway also regulates mitochondrial motility by phosphorylating and degrading Miro that anchors molecular motor to mitochondria and allows delivery of mitochondria to axons and synapses (Wang et al. 2011b).

CYTOPLASMIC CELL DEATH (TYPE III CELL DEATH)

The term necrosis has traditionally been used to describe an accidental form of cell death, usually resulting from a direct damage to the cell membrane. However, in the past 10 or 15 years, evidence began to mount that necrosis (or at least some forms of necrosis) may also be genetically programmed. This type of PCD unfortunately has not received the same attention as apoptosis and therefore is significantly less well understood.

The ability of a cell to decide about its own fate is so fundamental, that cells have several back-up programs for PCD, should one or two become disabled. The choice of cellular death program depends on the nature of cell death signal (intensity, duration), cell/tissue type, age, bioenergetic state of the cell, levels of intracellular calcium, and other factors (Oppenheim et al. 2001). Furthermore, the cell may trigger apoptosis but switch to necrosis should it become unable to carry out the apoptotic process. Cultured cells undergoing apoptosis will often die by secondary necrosis, similarly to a situation in which phagocytosis is blocked and apoptotic cells cannot be removed. Such cells will have features of both apoptotic and necrotic PCD. Similarly, when blocking apoptosis pharmacologically (caspase inhibition), or genetically (knocking out prodeath BAX and BAK, or overexpression of prosurvival BCL-2 or BCL-xL), cells will temporarily survive thanks to autophagy. However, once they break down most of the cellular substrates, death by necrosis will ensue (Lum et al. 2005).

The core characteristics of a cell that dies by necrosis are a rapid bioenergetic failure and loss of membrane integrity. This can be caused by opening of a “death channel” (Nishimura and Lemasters 2001), loss-of-function of ion channels, and/or damage to membrane lipids. This is followed by swelling of the cytoplasm and its organelles, membrane rupture and bursting of the cell. The end result is a release of cellular contents into the surrounding tissue and recruitment of inflammatory cells, which may in fact be advantageous in some types of sustained or excessive damage, and may play a role in disease.

Proteolysis during necrosis does not rely on caspases but on calpains and cathepsins. Although the different types of PCD are clearly distinct, they do have common components, at least in early phases of the death process and there may be a significant cross-regulation. For example, proapoptotic BCL-2 proteins (like BAX, BMF, BNIP3, or NIX) are also part of the necrotic program. For NIX, it is the subcellular localization that decides its role in apoptotic versus necrotic program (Chen et al. 2010). Often, activating one PCD pathway will block the others (Zong et al. 2004; O’Donnell et al. 2011). Please see the reviews by Bredesen et al. (2006), Baines (2010), and Peter (2011).

In this work, I will discuss two types of Type III cell death: necroptosis and parthanatos.

Parthanatos

This type of PCD depends on activation of poly(ADP-ribose)polymerase (PARP-1), which then directs translocation of AIF (released from intermembrane mitochondrial space at a later stage of apoptosis) to the nucleus (Yu et al. 2002). PARP-1 is a DNA repair enzyme that is typically induced by genotoxic stress and DNA damage, together with p53. The term “parthanatos” was first used by the Dawson group (Andrabi et al. 2008).

There is a good evidence of involvement of this pathway in PD. For example, neurons in PD patients show marked nuclear translocation of AIF (Burguillos et al. 2011). MPTP/MPP+ involves PARP-1 and AIF nuclear translocation (Mandir et al. 1999; Wang et al. 2003; Liou et al. 2005). Furthermore, dopaminergic neurons of Harlequin mice, which have 80%...
reduction of AIF, are sensitized to MPTP (Perier et al. 2010). And finally, PARP-1 inhibition prevents MPTP/MPP⁺-induced cell death (Cosi et al. 1996; Outeiro et al. 2007; Yokoyama et al. 2010), and α-synuclein cytotoxicity (Outeiro et al. 2007).

Necroptosis

Necroptosis is sometimes referred to as the “programmed necrosis” (Degterev et al. 2005) and can be described as an “ordered cellular explosion” (Vandenabeele et al. 2010). Necroptosis is linked to rapid mitochondrial dysfunction and an excessive ROS production. This programmed necrosis can be triggered on binding of ligands to the death receptors and is regulated by RIP family of kinases (reviewed by Vandenabeele et al. 2010). Data from genome-wide screens show that a BH3-only protein BMF is a component of the core necroptotic machinery (Hitomi et al. 2008) and that other regulatory proteins include cyclophilin D, PARP-1, and AIF (Galluzzi and Kroemer 2008; Declercq et al. 2009). Necroptosis can be blocked by pharmacologically inhibiting kinase activity of RIP1 by necrostatins (Degterev et al. 2005). However, the role of necroptosis in PD has thus far not been established.

CONCLUDING REMARKS

Understanding the intricate cellular mechanisms of PCD is a major step toward developing better disease-modifying treatments. In the past 20 years since the initial observation that MPP⁺ causes apoptotic cell death, we made significant progress in this regard. Our efforts have focused especially on mitochondrial dysfunction in PD because mitochondria have repeatedly been implicated in PD pathology and because mitochondria play a fundamental role in all three types of PCD.

One of the major breakthroughs in the field came with the identification of genes mutations that cause PD. Emerging data from genetic models (in vivo and in vitro) bring forward new processes and pathways that may be important for PD pathology. Some of these pathways are briefly outlined above. However, it is not clear if and how exactly they contribute to human PD. Neurons in PD may be dying because mutations in these genes may make them more prone to PCD, for example by negatively affecting the prosurvival function of these genes. One such example is regulation of the prosurvival Akt pathway by DJ-1 (Kim et al. 2005; Yang et al. 2005), Parkin (Fallon et al. 2006), and α-synuclein (Seo et al. 2002) (this topic is reviewed by Brunet et al. 2001; Burke 2008, 2010). However, many questions about the physiological roles of the proteins encoded by these genes still remain unanswered.

A large amount of resources were allocated to finding neuroprotective drugs that would halt the progression of dopaminergic cell loss. Unfortunately, this search has thus far been unsuccessful because the promising neuroprotection seen in models of PD failed to translate to the clinic (one possible exception may be rasagiline, however, there is an ongoing debate about the conclusiveness of the clinical data). There are several possible explanations for these disappointing results. First, there are several parallel, “back-up” PCD pathways and, therefore, cells may die by an alternative death pathway, should one PCD pathway become pharmacologically blocked. Cellular phenotypes of necrosis, apoptosis, and autophagy can indeed coexist in the same cell (Lemasters et al. 2002). In addition, cell soma and axon degeneration may be governed by different pathways (Bilsland et al. 2002; Raff et al. 2002; Ries et al. 2008; Cheng et al. 2011).

Another major obstacle in developing effective treatments has for a long time been the lack of animal models that would accurately reflect pathogenesis and cellular pathology of PD (reviewed by Waldmeier et al. 2006). Although displaying a robust cellular and behavioral dopaminergic phenotype, the most commonly used toxin-based model—MPTP mouse—may be such an example. Vertebrate genetic models of PD, on the other hand, do not typically show significant dopaminergic cell death. And while invertebrate genetic models (namely Drosophila or C. elegans) do display a strong dopaminergic phenotype (and evolutionary conservation of
PCD pathways together with the availability of an array of genetic tools makes them highly attractive for genetic screens), they are not very suitable for studying complex motor behaviors. The 6-OHDA rat model is useful for studies of axonal degeneration. However, among the different possible sites for 6-OHDA injections that cause parkinsonism, it is only the intrastriatal injection that causes the apoptotic type of cell death. In summary, many animal models of PD are available, each with their own shortcomings and it is therefore essential to be aware of advantages and limitations of each model.

Furthermore, the dose and schedule of application of a PD-mimicking toxin may have a profound effect on the type of cell death, as can be illustrated in the case of MPTP. The most commonly employed model follows an acute MPTP regimen (several doses in one day). Its advantage is that one can evaluate potential non-apoptotic mechanisms underlying cell death in response to the toxin, without too many confounding factors. Although more costly and with higher mortality rates, the sub-acute or chronic regimens are usually preferred because they are believed to more closely reflect the progressive nature of the disease process—for example, they allow for involvement of compensatory mechanisms the cells trigger in response to a moderate but sustained cellular damage. Moreover, chronic models allow a better assessment of neuroprotective effects (in acute models the tested drug is typically administered prior to the toxic insult, which is not at all what happens in the clinic). One of the potential issues with neuroprotective strategies may be that they start too late, after a majority of dopaminergic cell bodies and terminals are destroyed (which is when patients start to experience the classical motor symptoms). This may be too late for a neuroprotective intervention. Development of reliable biomarkers demonstrating disease progression prior to occurrence of the classical symptoms could therefore be therapeutically beneficial.

In summary, there are many signaling pathways regulating PCD and several types of PCD observed in PD. Therefore, we may need to develop drugs that concurrently modulate several PCD pathways. But most importantly, more time and resources need to be invested into basic research of neurodegeneration to gain more insight into pathogenesis of PD and molecular regulation of PCD. There is hope that, for example, genetic studies in PD patients, together with genetic screens in vitro and in small animal models of PD, will provide us with some answers.

REFERENCES

*Reference is also in this collection.*


K. Venderova and D.S. Park


Bossy-Wetzel E, Barsoum MJ, Godzik A, Schwarzenbacher K, Venderova and D.S. Park


Bossy-Wetzel E, Barsoum MJ, Godzik A, Schwarzenbacher K, Venderova and D.S. Park


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proapoptotic BH3-only BCL-2 family member, is critical for neuronal apoptosis. Neuron 29: 615–628.


Tatton NA, Kish SJ. 1997. In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience* **77**: 1037–1048.


Wang HL, Chou AH, Yeh TH, Li AH, Chen YL, Kuo YL, Tsai SR, Yu ST. 2007. PINK1 mutants associated with recessive...


Programmed Cell Death in PD


Programmed Cell Death in Parkinson's Disease

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