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DOI

[10.1016/j.molmed.2019.08.009](https://doi.org/10.1016/j.molmed.2019.08.009)

Publication date

2019

Document Version

Final published version

Published in

Trends in Molecular Medicine

License

Article 25fa Dutch Copyright Act

[Link to publication](#)

Citation for published version (APA):

Robinson, E. J., Aguiar, S., Smidt, M. P., & van der Heide, L. P. (2019). MCL1 as a Therapeutic Target in Parkinson's Disease? *Trends in Molecular Medicine*, 25(12), 1056-1065. <https://doi.org/10.1016/j.molmed.2019.08.009>

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Opinion

MCL1 as a Therapeutic Target in Parkinson's Disease?

Edward J. Robinson,¹ Sebastian Aguiar,² Marten P. Smidt,¹ and Lars P. van der Heide^{1,*}

Dopamine neurons in the substantia nigra (SN) pars compacta are selectively lost during the progression of Parkinson's disease (PD). Recent work performed on the role of the Bcl2 family (highly specialized proteins which control cellular survival and death) in midbrain dopamine neurons has led to the identification of the Bcl2 factor Mcl1 as a weak link in the survival of these neurons. We hypothesize that the regulation of BCL2 proteins may explain this selective vulnerability, and may even provide a novel therapeutic opportunity – strengthening weak links such as MCL1 could result in a delay or complete abrogation of cell death during PD.

Exposing the Intrinsic Vulnerability of Midbrain Dopamine Neurons

Underlying the motor symptoms of Parkinson's disease (PD) is the loss of dopaminergic neurons that project from the **substantia nigra (SN)**; (see [Glossary](#)) to the **striatum**. These neurons regulate voluntary movement, and their loss leads to tremor, progressive rigidity, loss of postural stability, and **bradykinesia** [1]. Current treatments are aimed at relieving clinical symptoms. There is no cure. The 'gold standard' in the treatment of PD is oral supplementation with **L-DOPA (l-3,4-dihydroxyphenylalanine)**, the blood–brain barrier-permeable precursor of dopamine (see Clinician's Corner). L-DOPA loses its efficacy over time and causes side effects such as L-DOPA-induced dyskinesias, which are suggested to be the result of conversion of L-DOPA to dopamine in serotonergic neurons by the enzyme aromatic l-amino acid decarboxylase (AADC) [2,3]. Halting the progressive loss of SN neurons would be a breakthrough in the treatment of PD. Although very promising results have been obtained in cellular and animal PD models, which display various degrees of neuroprotection, a clinical breakthrough is still lacking [4,5]. Interestingly, in both PD models and PD patients accumulating evidence points towards the mitochondria-dependent apoptosis pathway (Box 1) as the mechanism underlying neuronal loss [6,7]. This pathway is initiated when stressors activate specific proteins that permeabilize the outer mitochondrial membrane, resulting in the expulsion of toxic components that activate **caspases** and subsequently cause cell death [8,9]. Blocking apoptosis may therefore prevent the loss of dopamine neurons and represent a therapeutic approach [7]. Before a possible novel therapeutic target can be identified, it is crucial to understand which components of the apoptotic pathway are necessary to maintain **midbrain dopamine neurons**. Previously it has been hypothesized that specific transcription and growth factors orchestrate a set of dopamine neuron-specific Bcl2 factors to ensure proper dopaminergic development and maintenance [7] (Box 2). Bcl2 proteins are central to the apoptotic pathway because they can be classified as antiapoptotic or proapoptotic [9]. Their balance determines the fate of a cell. Recently, the antiapoptotic Bcl2 protein **Mcl1** has been identified as a crucial survival factor for midbrain dopamine neurons [10,11]. If the prosurvival function of BCL2 factors, and in particular MCL1, could be boosted therapeutically this would augment the resilience of dopaminergic neurons and possibly halt the loss of neurons in PD (see Clinician's Corner). The importance of Bcl2 factors in the midbrain dopamine system, the role of Mcl1 and its regulation, as well as possible PD therapeutic approaches will be discussed.

The Importance of Bcl2 Proteins in Dopaminergic Neurons

In one of the first studies addressing the role of Bcl2 factors (a list of the best-known Bcl2 factors is given in [Table 1](#)) in mouse dopamine neurons, a protective effect of Bcl2 expressed from the promoter of the gene (*Th*) encoding **tyrosine hydroxylase (Th)** was shown. Ectopic expression of Bcl2 resulted in an increased number of dopaminergic neurons and also protected against cell loss following quinolinic acid administration in the striatum, thus providing a proof-of-principle that Bcl2 factors can influence the resilience of dopaminergic neurons [12]. Several other BCL2 factors in addition to BCL2 itself have since been identified, mostly using cell lines with dopaminergic characteristics such as the

Highlights

The motor symptoms of PD are caused by loss of dopamine neurons in the SN of the brain. There is no cure, nor can the progressive loss of neurons be halted.

Several forms of regulated cell death have been attributed to this loss of dopamine neurons, of which apoptosis has emerged as a prominent candidate.

Mitochondria-dependent apoptosis is controlled by Bcl2 factors. Parkin, an E3 ligase, targets the proapoptotic Bcl2 protein Bax for degradation.

A reduction of Mcl1, an anti-apoptotic Bcl2 protein, in the *parkin* knockout mouse leads to loss of dopamine midbrain neurons.

Functional Mcl1 inhibition with a chemical compound results in apoptosis of neuronal cells and of dopamine neurons in the mouse SN.

Enhancing Mcl1 may be a therapeutic strategy to delay apoptosis of dopamine neurons in PD.

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Box 1. The Intrinsic Pathway of Apoptosis in PD

Apoptosis is a tightly controlled event that is necessary during development to eliminate cells that are wrongly located or incorrectly wired. Aberrant activation of this process underlies many neurodegenerative disorders. Broadly, apoptosis can be divided into two distinct pathways: the intrinsic mitochondrial pathway and the extrinsic pathway. The extrinsic pathway is activated by external signals that activate specific receptors, resulting in intracellular caspase activation. The intrinsic pathway can be activated by various signals including reactive oxygen species and DNA damage. BCL2 proteins play a crucial role in this pathway because they control mitochondrial outer-membrane permeabilization, a crucial step in intrinsic apoptosis. The cascade is activated when cellular stress signals activate BH3-only proteins. The role of BH3-only proteins is to activate the proapoptotic BAX and BAK either by direct binding or through antagonizing the antiapoptotic BCL2 proteins (Table 1). Translocation, insertion into the mitochondrial membrane, and oligomerization of these proapoptotic factors causes the formation of proteolipid pores in the outer mitochondrial membrane, through which apoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) can leak out, marking the point of no return. The release of cytochrome c leads to oligomerization of apoptotic protease-activating factor 1 (APAF1), eventually forming the apoptosome and activating the initiator caspase-9. Subsequent activation of executioner caspases such as caspase-3 then finally leads to the apoptotic phenotype in which essential cellular components are cleaved. Extensive evidence, ranging from post-mortem material from PD patients to animal models and cell-culture systems, indicates that intrinsic apoptosis plays a prominent role in the neurodegeneration observed in PD [6]. However, other forms of regulated cell death have also been described to play a role in PD. It is therefore possible that neuronal loss in PD may reflect various forms of cell death operating in concert (a recent overview on cell death mechanisms is given in [9]). Attenuating the intrinsic apoptotic pathway to halt neurodegeneration in PD patients thus requires advanced (genetic) diagnostics to confirm the suitability of the patient and to ensure successful treatment.

human SH-SY5Y cell line. Knockdown of MCL1 or BCL-xL decreased the viability of untreated SH-SY5Y cells, whereas knockdown of BCL2 had no apparent effect. However, upon application of various apoptotic stressors, such as daunorubicin, cisplatin, CCCP, and valinomycin, each individual knockdown (MCL1, BCL-xL, and BCL2) potentiated cell death [13]. The importance of BCL2 proteins

Box 2. Bcl2 in Dopamine Neurons: Mouse and Man**Bcl2 Proteins in the Mouse**

Given that Bcl2 proteins play an essential role in controlling cell death in many cell types, it is not surprising that they are also essential components of neurons. Bcl-xL and Mcl1 are expressed in mouse adult dopamine neurons [11,22,23,50]. *Mcl1* knockouts do not survive the implantation period after fertilization [51], and *Mcl1* knockouts used in the field are therefore usually conditional knockouts. Conditional knockout of *Mcl1* in the nervous system of the mouse leads to apoptosis of neuronal precursor cells in the forebrain [52]. In addition, *Bcl-xL*-deficient mice are also not viable and die at embryonic (E) day E13, with massive apoptosis of immature neurons and hematopoietic cells [53]. Mice with conditional knockout of *Bcl-x* specifically in catecholaminergic neurons (*Th-Cre x loxP-Bcl-x-loxP*) display reduced striatal dopamine neurons, as well as fewer Th-positive SNpc and locus coeruleus neurons, suggesting that Bcl-xL is important for the proper development of (among others) the SNpc [50]. A conditional double knockout of *Mcl1* and *Bcl-xL* leads to death of the complete nervous system at E14 [54]. Finally, *Bcl2* knockout mice show normal embryonic development but display lymphoid apoptosis, neuronal and intestinal lesions, and terminal kidney disease [55]. Taken together, these findings underline the importance of antiapoptotic Bcl2 proteins in the brain.

BCL2 Proteins in PD Patients

BCL-xL, BCL2 and BAX are expressed in human adult dopamine neurons [56–58], but no mutations or SNPs in genes encoding Bcl2 factors (to our knowledge) have been associated with PD, except for some of their regulators such as parkin. So far, studies have investigated BCL-xL in human control and PD patients. No differences in immunoreactivity could be detected in melanized SNpc neurons [56,57]; however, one study found an upregulation in BCL-xL mRNA in melanized dopamine neurons of PD patients compared with controls [58]. Moreover, no differences in BCL2 and BAX staining were observed in PD versus control patients [56,57].

Glossary

Agonists: chemicals that invoke an action response, in other words activators. By contrast, antagonists inhibit a response.

Bioenergetics: pathways which involve energy in organisms, such as growth, development, and metabolism. ATP is the main energy source used during these processes.

Bradykinesia: a slowness of movement that is characteristic of Parkinson's disease (PD) symptoms.

Caspases: specialized proteins that are known for their cleavage activity in the apoptosis pathway.

Degron: the part of a protein that is involved in the regulation of protein degradation. The best-described degrons are ubiquitin-dependent degrons that are involved in the polyubiquitination of a protein.

E3 ubiquitin ligase: a protein involved in the proteasome ubiquitination pathway. The E3 ubiquitin ligase facilitates the transfer of the ubiquitin groups from the E2 enzyme to the substrate.

Ubiquitination of a substrate often leads to degradation of the substrate by the proteasome.

Homozygous: homozygous loss of a gene means that both alleles are inactive, whereas heterozygous loss means that only one allele is inactive.

L-DOPA: L-3,4-dihydroxyphenylalanine, the precursor for the important neurotransmitter dopamine. Current treatment for alleviating PD symptoms is L-DOPA supplementation.

Midbrain dopamine neurons: neurons in the main dopaminergic areas of the midbrain that are often studied in relation to PD. These areas include the ventral tegmental area and the SN.

Mitophagy: the removal of damaged mitochondria by autophagy. This is an essential process because accumulation of damaged mitochondria leads to cellular degeneration.

MCL1/Mcl1; MCL1/Mcl1: all uppercase letters indicate a protein of human origin, whereas lowercase indicates the mouse protein; gene names are given in italics.

Peptides: short chains of amino acids, arbitrarily distinguished

Table 1. Classification of Bcl2 Factors

Class	Factor	Function	Refs
Antiapoptotic	Bcl2	Inhibition of proapoptotic Bcl2 factors Cell survival	[9,55]
	Bcl2A1	Inhibition of proapoptotic Bcl2 factors Cell survival	[8]
	Bcl-xL	Inhibition of proapoptotic Bcl2 factors Cell survival	[8,9]
	Bcl-w	Inhibition of proapoptotic Bcl2 factors Cell survival	[8]
	Mcl1	Inhibition of proapoptotic Bcl2 factors Cell survival	[8,9,51]
Proapoptotic	Bax	Oligomerization to induce MOMP ^a	[9]
	Bak	Oligomerization to induce MOMP	[9]
	Bok	Ability to induce MOMP in the absence of Bax and Bak	[9]
Proapoptotic BH3-only	BNIP3	Mitophagy	[21]
	BNIP3L	Mitophagy	[19]
	Noxa	Sequestration of antiapoptotic factor Mcl1	[9,59]
	Puma	Sequestration of antiapoptotic Bcl2 factors	[9,60]
	Bad	Sequestration of antiapoptotic factors Bcl-xL, Bcl2, and Bcl-w	[9]
	Bid	Sequestration of antiapoptotic Bcl2 factors Direct activation proapoptotic factors	[9]
	Harakiri	Sequestration of antiapoptotic factor Bcl-xL	[9]
	Bim	Sequestration of antiapoptotic Bcl2 factors Direct activation proapoptotic factors	[9]
	Bmf	Sequestration of antiapoptotic factors	[9]

^aAbbreviation: MOMP, mitochondrial outer-membrane permeabilization.

in the regulation of neuronal survival in these studies is apparent. Importantly, a causal relationship between PD and BCL2 factors has been established via the **E3 ubiquitin ligase parkin** [14,15]. Parkin is best known for its role together with the kinase PTEN-induced kinase 1 (PINK1) in the removal of damaged mitochondria via a process called **mitophagy** [16]. However, parkin has also been described to directly interact with and ubiquitinate Bax in mouse embryonic stem cells, preventing mitochondrial Bax translocation and thus preventing cell death in response to stress [14,17]. Interestingly, the R275W parkin mutation, which is associated with PD, failed to inhibit mitochondrial Bax translocation *in vitro*, thus providing a potential explanation for the excessive cell death observed in PD [17]. Conversely, the function of parkin and PINK1 can also depend on Bcl2 proteins. In fact, parkin-dependent mitophagy can be antagonized by prosurvival Bcl2 members by preventing its translocation to depolarized mitochondria [18]. Accordingly, parkin translocation to depolarized mitochondria is enhanced by BH3-only proteins [18]. In addition, the BH3-only Bcl2 proteins BCL2 interacting protein (BNIP)3 and BCL2 interacting protein 3 like (BNIP3L) have both been attributed different functions ranging from regulating PINK1 proteolytic cleavage to functioning as essential downstream substrates of parkin in the regulation of mitophagy [18–21]. Taken together, accumulating evidence suggests that Bcl2 proteins, and especially their specific regulation in midbrain dopamine neurons, might play a pivotal but complex role in the survival of these neurons in health and

from proteins at the maximum length of 50 amino acids.
Post-translational modifications (PTMs): modifications to a protein after translation, which (among others) include phosphorylation, cleavage, and ubiquitination.
Proteasomes: specialized protein complexes that can recognize ubiquitin-tagged proteins for degradation by proteolysis.
Small-molecule inhibitors: small organic compounds with a low molecular weight (<900 Da) which can influence the function and/or activity of the target.
Striatum: an area in the brain that receives innervation from dopaminergic neurons of the SN.
Substantia nigra (SN): a wing-shaped structure in the midbrain that contains dopaminergic neurons. Dopaminergic neurons of the pars compacta area of the SN are lost in PD.
Tyrosine hydroxylase (Th): the rate-limiting enzyme in the dopamine production. Catalyzes the conversion of L-tyrosine to the dopamine precursor L-DOPA.

disease (Figure 1A,B). However, from these studies it is unclear which BCL2 factor might potentially be used as a therapeutic target.

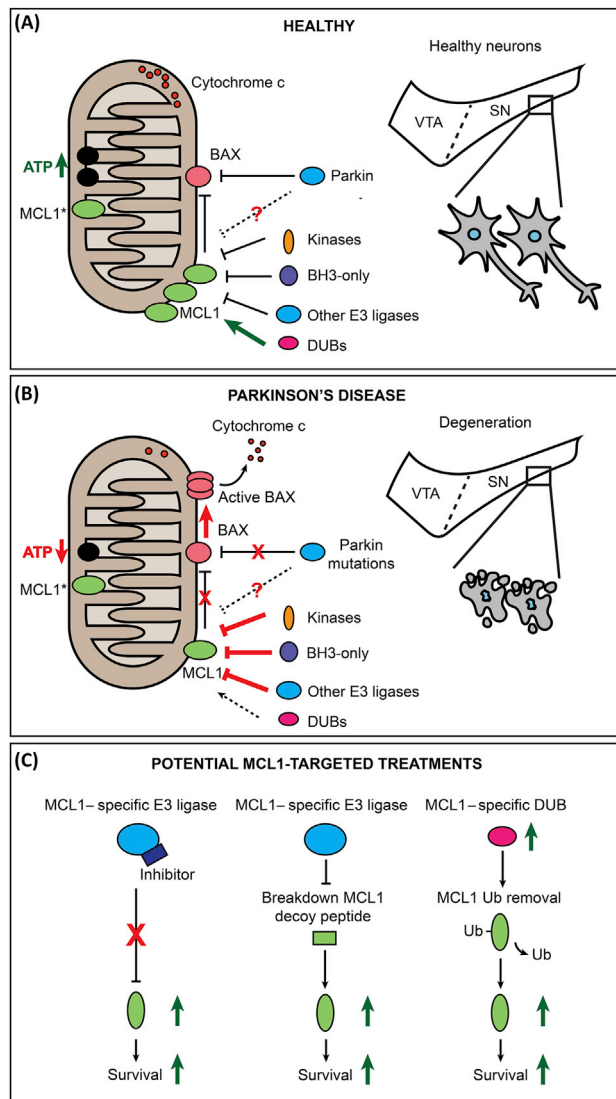
Mcl1 as a Survival Factor in Dopamine Neurons

Previous studies have shown that Mcl1 is highly expressed in neurons of the developing brain and that its expression drops dramatically in the adult, suggesting a role of lesser importance at this stage [22]. In contrast to Mcl1, the prosurvival Bcl2 factor Bcl-xL remains highly expressed in adult neurons and has been shown to be crucial for the survival of cortical neurons [22,23]. However, despite its overall low expression in the adult brain, recent studies support an important role for Mcl1 in the survival of adult dopamine neurons. Lu and colleagues studied the protein levels of Mcl1 and Bax in the striatum after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment [24]. MPTP treatment leads to cell death in the SN and has often been used to investigate PD. The expected reduction in dopaminergic terminals after MPTP treatment was accompanied by a 37% decrease in Mcl1 protein levels, whereas Bax levels remained the same [24]. Because dopaminergic terminals are abundant in the striatum, they investigated the levels of Mcl1 in that area rather than in the SN, although it would be interesting to look at Mcl1 protein levels in the SN after MPTP treatment.

Microarray and reverse transcriptase (RT)-qPCR data recently confirmed relatively high expression levels of *Mcl1* and *Bcl-xL* (*Bcl2l1*) in dopaminergic neurons of embryonic mice and newborns at post-natal day 2 [10]. By utilizing various **small-molecule inhibitors** of Bcl2 function, it was established that Mcl1, but not Bcl-xL, is of crucial importance for the survival of dopaminergic cells. Treatment of MN9D dopaminergic cells as well as cultured midbrain slices with the specific Mcl1 inhibitor UMI-77 resulted in an increase in the activation of the apoptotic marker, cleaved caspase 3. Moreover, functional inhibition of Mcl1 leads to increased Bax activation, which could be prevented by preincubation with a Bax-inhibiting **peptide** [10]. Taken together, the data suggest that both the function and levels of Mcl1 are important.

The importance of Mcl1 in the survival of dopaminergic neurons has recently been supported by others. Mice with **homozygous** deletion of *parkin* display an upregulation of Mcl1 protein, but no apparent cell death [11]. Additional removal of one allele of *Mcl1* in mice leads to progressively worse performance in several motor-skill performance tests during their first year after birth. Immunostaining of the midbrain dopaminergic system of these mice ($n = 3$) after 52 weeks revealed a dramatic loss of Th-positive neurons. Although preliminary, because neuronal quantification was performed in only three subjects per group, the authors suggest that upregulation of Mcl1 in the *parkin* knockout is a form of compensation, whereas the additional removal of one *Mcl1* allele abrogates this compensation and leads to a mouse which displays neurodegeneration of midbrain dopamine neurons [11]. Additional follow-up experiments at different timepoints will be necessary to gain more insight and confirm the suggested progression and magnitude of the neurodegeneration.

In addition to having a crucial role in apoptosis by inactivating proapoptotic Bcl2 factors, Mcl1 has also been implicated in mitochondrial fusion and oxidative phosphorylation [25,26]. Full-length Mcl1 encodes a 40 kDa protein, which can be proteolytically cleaved to yield a 38 kDa and 36 kDa product. Whereas localization of the 40 kDa and 38 kDa Mcl1 protein to the outer mitochondrial membrane is crucial for preventing apoptosis, the 36 kDa version is imported into the inner mitochondrial membrane and regulates mitochondrial **bioenergetics** but not apoptosis [26]. The underlying mechanism has been suggested to be the regulation of mitochondrial DNA content and inner mitochondrial membrane structure by Mcl1, as well as the correct assembly of F_1F_0 -ATP synthase oligomers [26]. It has been suggested that the bioenergetic demands of SN dopaminergic neurons are extremely high owing to the large scale of its axonal arborizations and terminal numbers [25]. Reducing the axonal arbor size of SN neurons *in vitro* with semaphorin 7A to levels comparable with neurons of the ventral tegmental area (VTA) reduced basal levels of oxidative phosphorylation and sensitivity to toxins such as rotenone and 1-methyl-4-phenylpyridinium (MPP+) [25]. Aberrant regulation of Mcl1 may therefore have disastrous consequences for cellular survival as well as for mitochondrial bioenergetics, and may even underlie part of the selective vulnerability of SN neurons observed in PD.



Trends in Molecular Medicine

Figure 1. The Pivotal Role of MCL1 in Dopaminergic Midbrain Neurons.

(A) In healthy neurons, MCL1 is abundant and prevents BAX from forming proteolipid pores in the outer mitochondrial membrane. BAX in turn can be ubiquitinated by parkin to ensure that pores do not form. MCL1 stability and levels are regulated by kinases, BH3-only proteins, E3 ubiquitin ligases (e.g., parkin, although this might be indirect), and deubiquitinases (DUBs). Moreover, MCL1 can be imported into the mitochondria (MCL1*) where it regulates the bioenergetics of the neuron. (B) Various stressors could influence the regulators of MCL1, which in turn negatively regulate the stability and/or function of MCL1 such that MCL1 can no longer inhibit BAX. In addition, parkin loss-of-function mutants can no longer degrade BAX, which then forms proteolipid pores through which cytochrome c can leak out and start the apoptotic pathway. In parallel, less MCL1 in the mitochondria negatively influences the bioenergetics of the neuron, potentially making it more vulnerable. (C) Because of its pivotal role, MCL1 might provide an interesting therapeutic target. Inhibition of a MCL1-specific E3 ligase or ‘tricking’ that particular ligase into breaking down the decoy peptide both lead to increased MCL1 levels and would boost the survival of the neurons. In addition, boosting the activity of a MCL1-specific DUB would also lead to increased MCL1 levels and increase survival. Abbreviations: SN, substantia nigra; Ub, ubiquitin; VTA, ventral tegmental area.

Targeting Mcl1: The Ubiquitin Proteasome System, E3 Ubiquitin Ligases, and Deubiquitinases

Mcl1 harbors a large N-terminal region packed with regulatory motifs. Interestingly, many of these motifs influence the stability of Mcl1. Mcl1 is a short-lived protein and is rapidly degraded, which is attributed to the ubiquitin **proteasome** system [27,28]. Depending on the cell type studied, Mcl1 half-life ranges from 1 h to 5 h. Human MCL1 contains 12 lysine residues, of which five are described to be extensively involved in its degradation: K5, K40, K136, K194, and K197 [29]. By comparison, mouse Mcl1 also contains 12 lysine residues, but only three have been experimentally confirmed to be involved in ubiquitination: K117, K175, and K178. These sites are conserved between humans and mice, and correspond to K136, K194, and K197, respectively, in humans [29,30]. However, the E3 ubiquitin ligase(s) that control Mcl1 in the midbrain dopaminergic system is unknown. Identifying and targeting this specific E3 ubiquitin ligase could potentially open up novel therapeutic approaches to treat PD.

To date, seven E3 ubiquitin ligases have been experimentally identified to mediate Mcl1 degradation: Mcl1 ubiquitin ligase E3 (MULE/Huwe1), β -TrCP, F-box w7 β (Fbw7 β), APC/Ccdc20, tripartite motif-containing 17 (Trim17), parkin, and membrane-associated ring-CH-type finger 5 (MARCH5) [13,29–36]. The degradation of Mcl1 leads, in most cases, to apoptosis which can be prevented by applying proteasome inhibitors such as MG132 or lactacystin in various cell models [28,37]. Carroll and colleagues showed that MCL1 can be tagged for degradation by the E3 ubiquitin ligase parkin in HeLa cells [13]. However, loss of parkin protein function would in theory lead to more Mcl1 [11], which in turn would protect the cell from undergoing apoptosis. Although parkin is an ideal candidate given its involvement in PD, it is less likely that parkin is involved in the direct breakdown of MCL1 in midbrain dopaminergic neurons (Figure 1B). Others have suggested that, upon stress, parkin targets the ubiquitin ligase SCF substrate adapter Fbw7 β , resulting in stabilization of Mcl1 and allowing neurons to survive [32]. Ubiquitination is a reversible event facilitated by deubiquitinases (DUBs), and a few Mcl1 DUBs have been identified. Indeed, USP9X/USP24 promotes cell survival by removing K48-linked polyubiquitin chains from Mcl1, which normally mark the protein for proteasomal degradation [38,39]. A third DUB, USP13, has recently been identified and was shown to control MCL1 stability by deubiquitination in human lung and ovarian cancer cells [40]. The DUB that potentially regulates MCL1 in midbrain dopaminergic neurons is unknown.

Targeting Mcl1 with Small Molecules and Peptides as a Therapeutic Approach

The BCL2 family has been the focus of drug discovery efforts in oncology, with the objective of inhibiting the protective guardian BCL2 family members to induce apoptosis in tumors. To our knowledge, no drug discovery or rational drug design effort has aimed to achieve the opposite – the selective enhancement of antiapoptotic BCL2 ‘guardian’ function to protect death-prone cells.

Small Molecules

The first approach to selectively enhance antiapoptotic BCL2 activity would be to develop inhibitors of enzymes that conjugate BCL2 guardians with **post-translational modifications (PTMs)** that are prodegradative, which include ubiquitination [41]. The known PTMs regulating MCL1 stability are listed in Table 2. In addition to the challenge of selectively inhibiting the target, off-target effects are another layer of risk: kinases, phosphatases, E3 ubiquitin ligases, ribosyltransferases, lipidating enzymes, and other enzymes mediating cell signaling are known to be promiscuous – acting on many downstream targets. Granted, if the inhibitor can be delivered selectively to particular cell types such as dopaminergic neurons in the substantia nigra pars compacta (SNpc), the risk of off-target effects may be acceptable – for example, inhibitors of an E3 ubiquitin ligase that is specific for MCL1 or other BCL2 family guardians that act specifically in the SNpc (Figure 1C).

A popular recent approach to PD treatment development has focused on inhibition of the DUB USP30, which antagonizes parkin-mediated mitophagy. Parkin is the ‘on’ switch and USP30 is

Clinician’s Corner

In PD, midbrain dopamine neurons are progressively lost; the typical clinical phenotype is visible after considerable neuronal loss. Most cases are sporadic and have no clear genetic basis.

The current gold standard treatment relies on supplementation with precursors of dopamine (L-DOPA), dopamine receptor agonists, and dopamine degradation enzyme inhibitors. This provides the patients with temporary symptom relief. However, this treatment loses its effectiveness over time and can cause L-DOPA-induced dyskinesias. Researchers have been trying to understand the molecular details of dopaminergic neurons and explain their apparent selective vulnerability – why is the SN pars compacta particularly vulnerable to cell death? Unique aspects of dopaminergic neurons include factors that are directly involved in decisions concerning cell survival, as well as reliance on members of the BCL2 family.

A promising therapeutic target is MCL1, a protective ‘guardian’ BCL2 family member that prevents cell death. By targeting interactors of MCL1 it may be possible to stabilize MCL1 in dopamine neurons of the SN. The identification of midbrain dopamine neuron-specific MCL1 regulators would open up a therapeutic opportunity for a disease-modifying treatment for PD.

Table 2. Post-translational Modifications of Mcl1

Modification	Residue	Description ^a	Refs
Ubiquitination	K5	Ub by MULE/HUWE1, proapoptotic	[29]
	K40	Ub by MULE/HUWE1, proapoptotic	[29]
Truncation	I10–G11	Yields a 38 kDa isoform that is loosely localized to the OMM	[26]
	L33–V34	Yields a 36 kDa isoform that is localized to the matrix.	[26]
Phosphorylation	S64	CDK1/2 and JNK site. Strongly phosphorylated in G2/M phase. Increased affinity for proapoptotic BCL2 proteins, but no effect on MCL1 stability.	[61]
	T92	Phosphorylated in tandem with T163 by ERK-1, increases stability. Requires Pin-1.	[62]
	S121	Phosphorylated in tandem with T163 by ERK-1, but conflicting results regarding stability after H ₂ O ₂ or TNF administration.	[63,64]
	S155	GSK3-β site, destabilizing degron for β-TrCP	[31]
	T156	Predicted, unknown effect	[65]
	S159	GSK3-β site, destabilizing degron for β-TrCP. Must be primed by phosphorylation at T163 by JNK. Also inhibits binding to Bim.	[66,67]
	S162	S162A mutation resulted in nuclear localization, decreased stability, reduced Bak interaction, and increased apoptosis.	[68]
	S163	Highly conserved ERK-1 motif; effects on stability and apoptosis depend on other types of phosphorylation and results are conflicting.	[69]
Cleavage	D127–G128 and D157–G158	Cleaved by caspase-3, removing the PEST region. The remaining BH1, 2, 3 fragments did not induce apoptosis, unlike the cleaved forms of Bcl2 and Bcl-xL.	[70]

^aAbbreviations: GSK, glycogen synthase kinase; OMM, outer mitochondrial membrane; PEST, region rich in proline (P), glutamic acid (E), serine (S), and threonine (T); TNF, tumor necrosis factor; Ub, ubiquitination.

the ‘off’ switch, removing ubiquitin from mitochondria destined for mitophagy [42]. Small-molecule inhibitors of USP30 are in development [43]. Likewise, such an approach may be viable for the BCL2 family – by inhibiting the DUBs that act upon the proapoptotic BCL2 factors. Proapoptotic BCL2 factors such as BIM, NOXA, and BAX have been shown to be targeted by E3 ubiquitin ligases such as β-TrCP [44], MARCH5 [35], and parkin [14], respectively. Because ubiquitination can be a reversible process, it is reasonable to think that these proapoptotic factors are also DUB targets. Indeed, the DUB USP27x has been shown to stabilize BIM and increase apoptosis [44], whereas the DUB UCH-L1 stabilizes NOXA [45], but their relevance to dopamine neurons is unknown [44,45]. Conversely, DUBs that stabilize MCL1 may be a target for allosteric activation (Figure 1C).

Other approaches include the development of **agonists** of BCL2 family members that stabilize interaction with BAK/BAX or the BH3-only proteins. The guardian BCL2 family acts by preventing the proapoptotic BCL2 family-members from permeabilizing the outer mitochondrial membrane. It may be worth promoting protein–protein interactions between the guardians and the proapoptotic proteins they sequester. The opposite has been achieved in the oncology field by BH3 mimetics that can displace proapoptotic proteins from antiapoptotic proteins. Interestingly, an effort by Garner and

colleagues to inhibit BAX led to a new class of small-molecule BAX inhibitors (BAIs). Instead of acting as a BH3 mimetic, BAIs bind to a unique BAI binding site and allosterically inhibit the activation of BAX by stabilizing inactive BAX [46].

Peptides

Achieving selectivity with small molecules is a perennial challenge. Peptides offer a solution. It may be possible to conjugate a potent neuroprotective peptide (naked or in a liposome) to an antibody or aptamer with avidity for dopaminergic markers present in dopamine neurons [47]. Wiley *et al.* demonstrated that a toxin-conjugated monoclonal antibody against the dopamine transporter DAT caused selective ablation of neurons in the SN, but not in other aminergic structures such as the raphe nucleus or locus coeruleus, when injected into the striatum or lateral ventricle [48].

One approach to boost neuronal survival is to develop 'decoy peptides' (which function as 'bodyguards') that feature the **degrons** of the endogenous protein to be protected (e.g., MCL1). By analyzing the degron sequences on MCL1, it may be possible to develop 5–10 amino acid peptide sequences that will take the prodegradative 'hit' PTM instead of the real protein (Figure 1C). Another approach is to mimic domains of BCL2 proteins or their interacting proteins. An example of such a peptide is the Bax-inhibiting peptide V5 (BIP-V5), a pentapeptide based on the Ku70–Bax interaction domain. BIP-V5 was neuroprotective in a neonatal mouse model of ischemia, and also prevented 6-hydroxydopamine (6-OHDA)-induced degeneration of SN dopamine neurons in the rat [49]. In addition, BIP-V5 prevented cell death induced by inhibition of Mcl1 with UMI-77 in MN9D dopaminergic cells [10].

Concluding Remarks

Halting the progressive loss of dopaminergic neurons in the SN is a major challenge in PD research. Causative mutations have been identified in a minor fraction of PD patients, but the majority of PD cases remain idiopathic with an identical end-result: neurodegeneration. By interfering with crucial components necessary for cell death, a generalized method for halting neurodegeneration in PD could be realized. Accumulating evidence supports a role for BCL2 proteins and mitochondria-dependent apoptosis in PD. The identification of Mcl1, a Bcl2 protein that prevents mitochondria-dependent apoptosis, could be of key importance in strategies designed to keep dopamine neurons alive [10]. Boosting MCL1 function by interfering with its ubiquitination and breakdown using small-molecule inhibitors or by preventing its processing with decoy peptides may be viable approaches. Before MCL1 function can be therapeutically boosted, several outstanding questions need to be resolved (see Outstanding Questions).

Acknowledgments

This work has been supported by Stichting ParkinsonFonds, The Netherlands.

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Outstanding Questions

Is preventing mitochondria-dependent apoptosis effective in preventing PD progression? Of the different forms of cell death related to PD, mitochondria-dependent apoptosis is the best described. However, it remains unknown whether blocking apoptosis is sufficient to prevent the known pathogenic mutations, mitochondrial dysfunction, and intracellular stress from killing dopaminergic midbrain neurons.

Is the prosurvival BCL2 protein MCL1 important for dopamine neuron survival in humans? Most data regarding the importance of Mcl1 have been acquired from animal or cellular work, and patient data are lacking. For example, it is not known whether MCL1 protein is expressed in the SN in humans, and whether it is the weakest link in the chain that actively suppresses apoptosis.

What is the MCL1 E3 ligase or DUB in midbrain dopamine neurons? Different E3 ligases and two DUBs have been identified in various cell types, but the identity of the relevant factors in human SN is not known.

What is the best way to therapeutically target MCL1 in dopamine neurons and ensure the highest specificity and lowest off-target effects? Targeting SN dopamine neurons poses a real challenge and would ideally make use of a marker that is uniquely present in this area. In addition to the uniqueness of the target, the delivery method of the small molecule or peptide needs to be investigated thoroughly to ensure optimal delivery.

Which BCL2 factor is rate-limiting for survival after MCL1? Enhancing MCL1 function and the machinery around it could possibly lead to another (BCL2 family) factor becoming rate-limiting. To solve this, the entire repertoire and interactome of BCL2 factors in human SN neurons needs to be mapped.

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