Regulation of neuronal autophagy and cell survival by MCL1 in Alzheimer's disease

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ABSTRACT

Maintaining neuronal integrity and function requires precise mechanisms controlling organelle and protein quality. Alzheimer's disease (AD) is also characterized by functional defects in the clearance and recycling of intracellular components. In fact, neuronal homeostasis involves autophagy, mitophagy, apoptosis, and compromised activity in these cellular processes may cause pathological phenotypes of AD. Therefore, mitophagy is a critical mitochondrial quality-control system, and impaired mitophagy is a hallmark of AD. Myeloid cell leukemia 1 (MCL1), a member of the pro-survival B-cell lymphoma protein 2 (BCL2) family, is a mitochondrially targeted protein that contributes to maintaining mitochondrial integrity. *Mcl1*-knockout mice display peri-implantation lethality. Studies on conditional *Mcl1*-knockout mice have demonstrated that MCL1 plays a central role in neurogenesis and neuronal survival during brain development. Accumulating evidence indicates the critical role of MCL1 as a regulator of neuronal autophagy, mitophagy, and survival. In this review, we discuss the emerging neuroprotective function of MCL1 and how dysregulation of MCL1 signaling is involved in the pathogenesis of AD. Because members of the pro-survival BCL2 family proteins are promising targets of pharmacological intervention with BH3 mimetic drugs, we also discuss the promise of MCL1-targeting therapy in AD.

Keywords: Alzheimer's disease, mitochondria, autophagy, mitophagy, apoptosis, MCL1, BH3 mimetics

1. INTRODUCTION

1.1 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, accounting for an estimated 60–80% of dementia cases [1]. AD is a slowly progressing, multifactorial neurodegenerative disorder characterized by memory loss, cognitive impairment, and changes in behavioral function [2]. Most patients develop AD after the age of 65 years, whereas less than 5% experience rare early onset below the age of 65. Moreover, approximately 1% of cases or less develop AD because of autosomal-dominant genetic inheritance of mutations affecting the gene function of amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2), thus resulting in remarkably early onset and rapid progression of symptoms [3–5]. An estimated 6.2 million Americans 65 years of age or older were affected by AD in 2020. This number is estimated to continue to increase to approximately 88 million by the middle of the 21st century [1]. The symptoms of AD generally progress adversely during daily activities over the disease

course and eventually become fatal after the onset of AD symptoms. In the US, AD is currently the 6th leading cause of death [1]. Despite the high incidence of the disease, AD currently has no effective cure, because of the lack of knowledge regarding the molecular mechanisms underlying AD pathogenesis and disease progression. Therefore, therapeutic treatments for AD are urgently needed. To this end, the Food and Drug Administration has approved four drugs: cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and N-methyl-D-aspartate receptor antagonists (memantine). However, these drugs show only modest effects in ameliorating symptom progression and do not prevent worsening cognitive impairment [6]. Therefore, improved understanding of AD pathogenesis mechanisms is essential to prevent disease development and progression, and develop effective therapeutic interventions.

1.2 Pathology of Alzheimer's disease

The characteristic neuropathological hallmark of AD is senile plaques and neurofibrillary tangles (NFTs), which may subsequently cause neuronal degeneration and

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death [7, 8]. Senile plaques occur primarily through extracellular deposition of β -amyloid ($A\beta$) in the medial temporal lobe and the cortical layer of the hippocampus in the brain. $A\beta$ is produced from APP through sequential processing by β -secretase and γ -secretase [9]. Mechanistically, β -secretase cleaves APP at its N terminus, thus producing the membrane-bound APP fragment C99, which undergoes sequential γ -secretase-mediated processing [10]. This cleavage liberates 42- and 40-amino-acid fragments from the C99 N terminus, denoted $A\beta$ 42 and $A\beta$ 40, respectively, into the extracellular space. Because of its hydrophobicity, $A\beta$ 42 facilitates pathological aggregation, which in turn causes neuronal lethality and brain amyloidosis.

Beyond the $A\beta$ 42 filaments observed in senile plaques, NFTs are another neuropathological characteristic of AD. NFTs form from twisted Tau protein fibers and accumulate in vulnerable neurons. Tau, primarily expressed in the brain, is a microtubule-associated protein whose physiological function is to stabilize microtubules of neuronal cells [11]. In the AD brain, the hyperphosphorylated and misfolded state of Tau protein facilitates Tau aggregation and the formation of intracellular tangles. Although the underlying mechanisms leading to pathological Tau aggregation remain to be identified, the Tau-neurofibrillary pathology is closely correlated with the progression of cognitive decline in people with AD [12], and the spreading of the Tau filament from the entorhinal cortex to the neocortex is believed to be causally linked to cognitive impairment in people with AD [13]. Therefore, these two major pathological lesions are the primary targets for therapies to prevent AD onset and halt AD progression.

2. AUTOPHAGY IN ALZHEIMER'S DISEASE

2.1 Autophagy

Autophagy is an evolutionarily conserved intracellular degradation and recycling process in eukaryotic cells. Autophagy occurs in response to various stresses, such as nutrient deprivation, growth-factor depletion, infection, and inflammation. Autophagy was considered a process of non-selective degradation and recycling of a random cytoplasmic fraction, known as bulk autophagy, which occurs under nutrient- or energy-depletion stress conditions. Accumulating evidence indicates that autophagy also exerts critical cytoprotective roles by selectively removing unwanted and harmful cytoplasmic materials, such as protein aggregates and damaged organelles, in a process currently known as selective autophagy.

Autophagy is categorized into three types in mammalian cells—microautophagy, chaperone-mediated autophagy, and macroautophagy—according to the mechanism of how target substrates are delivered to lysosomes for degradation [14, 15]. In microautophagy, the lysosomal membrane invaginates and directly engulfs cytoplasmic cargo. In contrast, chaperone-mediated

autophagy uses transportation of cytoplasmic proteins to lysosomes with chaperones, that is further recognized by the receptor lysosomal-associated membrane protein 2A (LAMP-2A) on the lysosomal membrane, thus resulting in direct engulfment by lysosomes. Macroautophagy involves double-membrane-bound intermediary vesicles called autophagosomes, which engulf cytoplasmic components and organelles, and subsequently fuse with lysosomes and forming autolysosomes, which break down the encapsulated contents. Because autophagic processes are mediated primarily through macroautophagy, macroautophagy is regarded as a major autophagic pathway and therefore is referred to as autophagy hereafter. Under normal conditions, autophagy helps maintain cell functions through the specific degradation of damaged or excess organelles, such as overloaded peroxisomes (perophagy) and dysfunctional mitochondria (mitophagy). Under stress conditions, such as nutrient and energy starvation, cells accelerate the autophagic pathway, which breaks down cytoplasmic materials into metabolites that can be further used for biosynthetic processes and energy production to maintain cell survival.

Mechanistically, in response to energy deprivation, the activation of AMP-activated kinase (AMPK) and suppression of mechanistic target of rapamycin complex 1 (mTORC1) induce the transcriptional activity of transcription factor EB (TFEB) and consequently increase the transcription of autophagy-related genes (ATGs) and lysosome-associated genes [16]. AMPK also activates the autophagy-induction complex Atg/Unc52-like kinase 1 (ULK1), partly through phosphorylating ULK1 [17]. The activated ULK1 complex induces nucleation of the phagophore by phosphorylating Beclin1 [18], a component of the class III PI3K (PI3KC3) complex I, also referred to as the VPS34 complex (consisting of class III PI3K, Beclin1, VPS34, and ATG14) [19–21], which catalyzes PI3P formation. ULK1 also phosphorylates ATG9, which in turn recruits PI3P-binding proteins, such as WD-repeat-domain phosphoinositide-interacting proteins to the phagophore [22], thus leading to further recruitment of the ATG12-5-16L complex. This complex recruits the microtubule-associated protein light chain 3 (LC3) to PI3P-positive membranes, thereby promoting LC3 lipidation, and facilitating membrane elongation and target-protein recognition [22]. After autophagosomes form, they fuse with lysosomes, forming autolysosomes, which initiate the degradation of macromolecules (Figure 1A).

Neuronal cells are terminally differentiated and post-mitotic, and have high energy and nutrition demands; therefore, they are distinct from non-neuronal dividing cells. Maintaining the integrity of neuronal homeostasis is critical for cell survival during an individual's lifespan. Autophagy provides an effective system for the clearance and recycling of intracellular components, such as damaged organelles and aggregated proteins. Dysregulation of autophagy is

Figure 1 | Scheme of autophagy and mitophagy pathways.

(A) Nutrition or energy stress activates AMPK and suppresses mTORC1, thus leading to elevated ULK1 complex activity, which in turn promotes VPS34 and ATG5-12-16L complex formation, thereby facilitating phagophore synthesis and autophagosome formation. (B) Mitochondrial depolarization stabilizes PINK1 and activates PINK1/Parkin signaling, thus enhancing the phospho-ubiquitin conjugation of the OMM. The polyubiquitin chain is recognized by mitophagy receptors, such as OPTN and NDP52, and mitophagosomes subsequently form. Ubiquitin-independent mitophagy is mediated by BNIP3 and NIX mitophagy receptors. OMM: outer mitochondrial membrane.

considered a hallmark of AD [23, 24]. Aberrant accumulation of autophagosome vacuoles is observed in AD brains and transgenic mouse models, owing to the compromised fusion of autophagosomes with lysosomes [23, 25, 26]. However, the molecular mechanisms causing impaired autophagy function remain under investigation. One study has indicated that Beclin1 levels are diminished in AD brains; consistently, heterozygous knockout of *Beclin1* in hAPP-transgenic mice has been found to increase A β pathology [27]. In addition, hyperactivation of PI3K/mTORC1 signaling and suppression of the transactivation activity of TFEB are believed to be associated with impaired autophagy in AD. Mechanistically, mTORC1 negatively regulates autophagy by phosphorylating ULK1 and AMPK [28]. In support of this mechanism, clinical observations have indicated that the activity of mTORC1 and its downstream S6K is elevated in the brains of patients with AD [29, 30]. In contrast, TFEB is a positive autophagy regulator that induces expression of ATGs and lysosome-associated genes [31]. Notably, TFEB transcriptional activity is negatively controlled by mTORC1 kinase activity [32], thus implying that TFEB transcriptional activity is suppressed in mTORC1-activated AD neurons. Because autophagy is a critical neuronal protection system, further analyses are required to elucidate the molecular link between autophagy function and AD.

2.2 Mitophagy

In addition to toxic pathological protein aggregates, mitochondrial dysfunction is a major cause of neurodegenerative diseases such as AD [23]. Impaired mitochondrial function increases intracellular reactive oxygen species (ROS) levels, thus resulting in accumulating damage to various cellular components, including proteins, the genome, and lipids. Therefore, selective removal of dysfunctional mitochondria, a process referred to as mitophagy, is a critical protective mechanism to maintain cellular integrity. Mitophagy is driven primarily through the conserved PTEN-induced putative kinase 1 (PINK1)-Parkin signaling pathway in a ubiquitination-dependent manner [33, 34] (Figure 1B). PINK1 is an unstable protein kinase that is targeted to mitochondria and resides in the inner mitochondrial membrane (IMM), where it surveils mitochondrial integrity. Damaged or depolarized mitochondria lead to PINK1 stabilization and translocation to the outer mitochondrial membrane (OMM), and the subsequent promotion of PINK1 autophosphorylation and activation [35]. Subsequently, activated PINK1 phosphorylates both ubiquitin (Ub) on the OMM and the Ub-like domain of the E3 ligase Parkin, thus leading to Parkin's recruitment and activation [36, 37]. PINK1 and Parkin cooperatively modify OMM through PINK1-mediated phosphorylation of Ub (forming pUb) and Parkin-mediated conjugation of pUb chains on the

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proteins of OMM; consequently, a feed-forward loop forms, which enhances the modification of OMM with pUb-chains and provides binding platforms for autophagy receptors, such as optineurin (OPTN), nuclear domain 10 protein 52 (NDP52), and Tax1-binding protein 1 (TAX1BP1) [33, 38, 39]. Biologically, the binding of autophagy receptors to the OMM through interaction of the Ub-binding domain (UBD) with pUb subsequently leads to cargo binding through the interaction between LC3 and LC3-interacting region (LIR) motif of the autophagy receptors, thus promoting engulfment of the damaged mitochondria. TANK-binding kinase 1 (TBK1) facilitates this process through phosphorylating both UBD and LIR of the autophagy receptor OPTN, thereby enhancing the interaction of pUb-UBD and LC3-LIR, respectively.

The physiological and pathological importance of mitophagy has been further demonstrated by a study of *Pink1*- and *Parkin*-knockout mice. These mice are sensitive to mitochondrial stress, which promotes the release of damage-associated molecular patterns [40–42] and leads to innate inflammation [43]. However, further studies on the cross-talk mechanism between mitophagy and inflammation are needed to further elucidate the role of inflammation in the pathology of AD. Beyond the PINK1/Parkin-mediated Ub-dependent pathway, mitophagy is also induced by the Ub-independent pathway through the OMM-targeted mitochondrial mitophagy receptors BCL2, adenovirus E1B 19 kDa-interacting protein 3 (BNIP3), and BNIP3-like protein X (NIX) (Figure 1B). These proteins contain a mitochondrial-targeting motif and LIR domain, thereby facilitating the recruitment of autophagic vesicles to damaged mitochondria in a Ub-independent manner [44].

Mitophagy is a critical neuronal-protection process [45]. One study has reported defective mitophagy in the hippocampus in patients with AD, neurons derived from induced pluripotent stem cells from patients with AD, and an AD mouse model [46]. That study has demonstrated that mitophagy induction in APP/PS1-transgenic mice suppresses A β and phospho-Tau pathology, decreases levels of inflammatory cytokines, and ameliorates the cognitive-impairment phenotype. Another independent study has shown that AD neurons of hAPP-transgenic mice and AD brains display aberrant accumulation of mitophagosomes [47]. Beyond AD, Parkinson's disease (PD) and Huntington's disease are neurodegenerative diseases that also display progressive neuronal loss. They present a common pathogenic hallmark of misfolded protein aggregates and abnormal elimination of damaged mitochondria. Notably, genetic alterations in *Pink1* and *Parkin* cause early-onset PD [48]. In some forms of PD, PINK1 mutants fail to recruit Parkin to damaged mitochondria [49]. In addition, *Parkin* mutations associated with PD show a deficit in depolarization-insult-dependent translocation of Parkin to mitochondria [50]. Thus, aberrant PINK1/Parkin signaling causes dysregulation of mitophagy and subsequently leads to PD pathogenesis. Likewise,

dysregulation of mitophagy is a potential pathological feature in Huntington's disease [51]. In this scenario, the presence of mutant Huntington protein causes impaired cargo loading during mitophagy, as confirmed in striatal cells derived from knock-in mice carrying 111 CAG repeats in the *HTT* gene [52].

2.3 Mitochondrial dysfunction in AD pathology

Mitochondrial dysfunction is a characteristic feature of AD [23, 24, 53], which accelerates A β production in the brain in the AD mouse model [23, 54]. For instance, compromised mitochondrial function due to toxic insult or depletion of mitochondrial components enhances pathologic phenotypes of A β [55, 56]. Specifically, toxin exposure that causes mitochondrial damage promotes cognitive impairment and A β deposition in APP/PS1-transgenic mice. Heterozygous deletion of the mitochondrial antioxidant manganese *Superoxide dismutase-2* (*SOD2*) in hAPP-transgenic mice exacerbates the AD phenotype. These data suggest that dysfunctional or damaged mitochondria are a potential target for AD therapeutic intervention.

Impaired mitochondrial function is considered to promote APP processing to A β 42 and Tau hyperphosphorylation—two major pathological hallmarks of AD. Glucose uptake, the tricarboxylic acid cycle, oxidative-phosphorylation enzymatic activity, and mitochondrial biogenesis are diminished in AD, thus leading to decreased ATP and increased ROS levels, which may facilitate A β and Tau pathology [9, 57]. Decreased mitochondrial activity occurs before the accumulation of A β in the brain, as shown in several studies using an AD mouse model [24, 58, 59]. Mitochondrial dysfunction may result in overproduction of ROS and subsequently lead to aberrant APP processing through ROS-dependent upregulation of γ -secretase activity [60]. ROS overproduction causes pTau and NFT formation, and microtubule de-polymerization [61–64]. In contrast, the accumulation of A β and pTau reciprocally affects mitochondrial dysfunction [23, 65]. Moreover, the exposure of cultured neurons to A β /pTau and ectopic expression of mutant APP/Tau in mice lead to impaired mitochondrial function [65–68]. These findings suggest that the A β accumulation and Tau hyperphosphorylation affecting mitochondrial malfunction are exacerbated by impaired mitochondrial function.

3. MCL1 SIGNALING AND ALZHEIMER'S DISEASE

3.1 Regulation of the MCL1 pathway

MCL1 is a critical regulator of the cell-survival pathway. MCL1 is a member of the B-cell lymphoma protein 2 (BCL2) protein family, which controls cellular apoptosis through mitochondrial outer-membrane permeabilization [69]. According to function and structure, the BCL2 family is divided into three subgroups: multidomain pro-survival proteins (BCL2, MCL1, BCL-XL, BCL-W, and A1), multidomain pro-apoptotic effector proteins (BAK

and BAX), and BH3-only pro-apoptotic proteins (BID, BIM, PUMA, NOXA, BAD, BIK, BMF, and HRK). All family members contain a common BH3 polypeptide motif. In response to apoptotic stimuli, cells modulate the interaction status and protein levels of BCL2 family proteins toward releasing pro-apoptotic Bcl-2 antagonist killer 1 (BAK) and Bcl-2-associated X protein (BAX), thus promoting BAK and BAX homo-oligomerization, which in turn induces mitochondrial apoptotic pore formation and cytochrome c release [70] (Figure 2A).

MCL1 exerts its anti-apoptotic function by constraining pro-apoptotic BCL2 proteins through direct interaction. Distinctly from other anti-apoptotic BCL2 family proteins, MCL1 protein is extremely unstable, and rapid MCL1 degradation enables cells to promptly switch to a pro-apoptotic state (Figure 2B). MCL1 protein stability is tightly regulated by multiple post-translational

modifications including, but not limited to, phosphorylation, ubiquitination, SUMOylation, and acetylation. MCL1 has a unique extended N-terminal region with a PEST domain enriched in proline, glutamate, serine, and threonine, which are commonly found in proteins with short half-lives. Recent studies have revealed that MCL1 degradation is regulated partly through phosphorylation of the PEST domain by GSK3 β , JNK, p38, CK2, CDK1, and CDK5 [71-75] through proteasomal degradation. The ubiquitination of MCL1 is controlled by multiple E3 ligases, including MULE [76], SCF $^{\beta}$ -TRCP [77], APC/C Cdc20 [74], SCF FBW7 [72, 73], TRIM17 [78], TRIM11 [79], FBXO4 [80], and Parkin [81, 82]. Notably, SCF $^{\beta}$ -TRCP, SCF FBW7 , and APC/C Cdc20 require prior phosphorylation for MCL1 ubiquitination and degradation. In contrast, the proteasomal degradation of MCL1 is counteracted by multiple deubiquitinases (DUBs), including USP9X [83],

Figure 2 | MCL1-mediated regulation of apoptosis.

(A) The intrinsic apoptosis pathway is controlled by the interaction network of BCL2 family proteins. (B) MCL1 suppresses apoptosis by antagonizing BAX and BAK. After cytotoxic stimulation, MCL1 proteasomal degradation or binding to BH3-only proteins results in BAX/BAK oligomerization, thus leading to cytochrome c release.

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USP13 [84], OTUD1 [85], DUB3 [86], Ku70 [87], and JOSD1 [88]. Similarly to ubiquitination, SUMOylation regulates various cellular processes. MCL1 is a target of SUMOylation, which counteracts MCL1 ubiquitination, thus leading to MCL1 stabilization [79]. Furthermore, MCL1 has been reported to undergo acetylation [89]. In this scenario, the acetyltransferase p300 targets MCL1 for acetylation, which stabilizes MCL1 protein by facilitating its interaction with the DUB USP9X. Because genomic alterations in *MCL1* have not been reported in AD, post-translational modifications may be a potential MCL1-regulatory mechanism in this disease.

3.2 MCL1 function in the regulation of mitochondrial integrity

Beyond its pro-survival function, MCL1 plays an essential role in regulating non-apoptotic signaling governing mitochondrial integrity. MCL1 has been reported to control mitochondrial fission and fusion during the reprogramming of human pluripotent stem cells [90]. Mitochondrial fusion maintains mitochondrial network integrity, whereas mitochondrial fission facilitates the removal of damaged mitochondria through mitophagy. MCL1 contributes to these processes by directly interacting with two GTPases: DRP-1 and OPA-1 [90]. MCL1 binding to DRP-1 stabilizes DRP-1 protein at the OMM, thus leading to mitochondrial fission and subsequent degradation by mitophagy. In contrast, MCL1 destabilizes OPA-1 through interaction, repressing mitochondrial fusion. Furthermore, MCL1 contributes to mitochondrial calcium uptake through interaction with the OMM-localized voltage-dependent anion channel. This MCL1-dependent mitochondrial calcium uptake increases cell motility and mitochondrial ROS production [91]. The other roles of MCL1 in controlling mitochondrial activity are energy production and metabolism. The IMM-targeted isoform of MCL1 plays a critical role in mitochondrial bioenergetics through facilitating ATP synthase oligomerization and ATP production [92]. Moreover, IMM-localized MCL1 also regulates energy metabolism partly by promoting fatty-acid β -oxidation through binding the very long-chain acyl-CoA dehydrogenase (VLCAD) at the IMM [93]. These emerging MCL1 non-pro-survival functions may also contribute to AD pathology, because mitochondrial function is tightly associated with neuronal cell integrity and plasticity.

3.3 MCL1-dependent regulation of neuronal cell death

MCL1 is distributed in multiple tissues, including in the central nervous system. Because *Mcl1*-knockout mice show peri-implantation embryonic lethality [94], mice with heterozygous deletion and neural-progenitor-specific deletion have been used to interrogate the physiological role of MCL1 in protecting against neuronal cell death [95-97]. Heterozygous *Mcl1*-knockout mice show elevated apoptosis in response to excitotoxic insult in hippocampal pyramidal neurons [95]. MCL1 is highly

expressed in neural precursors within the ventricular zone and newly committed post-mitotic neurons within the developing cortical plate. Neuronal-progenitor-specific *Mcl1*-conditional-knockout mice show severe defects in cortical neurogenesis, owing to extensive cell death during nervous-system development, thus causing embryonic lethality at embryonic day 16. Cerebellar granule neurons (CGN) derived from *Mcl1*-knockout mice show elevated sensitivity to DNA-damage-induced apoptosis [96].

Several studies have focused on the molecular mechanisms of MCL1 regulation in neurons. In CGN culture, neuronal cellular apoptosis, induced by serum and potassium deprivation, has been found to be triggered by proteasomal degradation of MCL1 in a manner dependent on the TRIM17 E3 ligase [78] (Figure 3). TRIM17 has been reported to promote neuronal apoptosis [98, 99] and induce the expression of alpha-synuclein [100]. Deprivation of serum and potassium induces the sequential phosphorylation of Thr144 and Ser140 by JNK and GSK3 β , respectively. TRIM17 recognizes phosphorylated Ser140 and Thr144, corresponding to Ser159 and Thr163 in human MCL1, for proteasomal degradation. These sites comprise the recognition motif (phospho-degron) of FBW7, a tumor suppressor and substrate adaptor of the SCF^{FBW7} E3 ligase complex [72, 73], which is responsible for MCL1 degradation in various types of cancers and neuronal cells [101, 102]. Moreover, in the mouse primary CGN, the depletion of *TRIM17* results in MCL1 stabilization [78]. Phosphorylation-directed MCL1 degradation is also mediated by CDK5, which is highly expressed in neuronal cells; CDK5 activity is critical for neurogenesis and pathogenesis of AD [103, 104] (Figure 3). CDK5-mediated MCL1 Thr92 phosphorylation promotes proteasomal MCL1 degradation, thus increasing neuronal vulnerability and mitochondrial depolarization in glutamate-treated HT22 mouse hippocampal and primary cortical neuronal cells [75]. These findings may partly explain the molecular mechanisms of how CDK5 hyperactivation may cause neurodegeneration, beyond phosphorylating Tau protein [105]. Furthermore, the critical mitophagy regulator Parkin promotes MCL1 ubiquitination and degradation, and subsequently induces apoptosis of dopaminergic neurons [81]. Mitochondrial depolarization, but not pro-apoptotic stimulation, activates the PINK1/Parkin complex for MCL1 degradation. Moreover, Parkin regulates the FBW7-MCL1 pathway through Parkin-mediated FBW7 degradation in dopaminergic neurons; therefore, the activated PINK1-Parkin pathway indirectly stabilizes MCL1 and consequently promotes neuronal survival [106]. These studies together suggest a dual function of the PINK1/Parkin complex in regulating both mitophagy and apoptosis.

Although genetically engineered animal models have revealed the crucial role of MCL1 in regulating brain development, relatively little is known regarding MCL1's physiological function in AD pathogenesis.

Figure 3 | Phosphorylation-dependent regulation of MCL1 protein in neurons.

Phosphorylation facilitates MCL1 degradation. Withdrawal of serum and potassium, injury, or glutamate toxicity stimulation induces MCL1 phosphorylation, thus leading to rapid MCL1 degradation in a proteasome-dependent manner. GSK3 β plays a major role in phosphorylation at Ser140, thereby facilitating recognition by E3 ubiquitin ligases, such as FBW7 and TRIM17. MAPKs—including JNK, which is responsible for Thr144 phosphorylation—may serve as priming kinases for GSK3 β -dependent Ser140 phosphorylation. Phosphorylation at Thr92 by CDK5 also induces MCL1 proteasomal degradation by an unidentified E3 ubiquitin ligase. Rapid removal of MCL1 at mitochondria results in the induction of neuronal apoptosis.

Among the BCL2 family of proteins, the role of BCL2 in AD is relatively well defined. Evaluation of the abundance of BCL2 family members in AD has suggested that the shift in the balance between pro-survival and pro-apoptotic family proteins toward pro-apoptosis may facilitate disease progression. In support of this possibility, A β treatment in human primary neuronal culture has been found to downregulate BCL2 protein levels and upregulate BAX levels [107]. In agreement with this finding, A β injection into the mouse hippocampus increases Bim levels and decreases BCL2 levels, thus leading to BAX oligomerization and subsequent neuronal cell death [108]. In addition, A β deposition in an AD mouse model has been found to upregulate miR-16-5p, which targets BCL2 expression and consequently decreases BCL2 protein levels [109]. In agreement with these observations, MCL1 protein levels have been negatively correlated with disease severity in AD brains [75]. A study using the 3 $\times$ Tg AD mouse model has revealed the critical function of BCL2 in suppressing AD pathogenesis [110]. BCL2 overexpression in mice limits caspase-9 activity and decreases NFT and A β plaque formation; consequently, the mice show ameliorated cognition impairment [110]. These findings further define the physiological pro-survival function of BCL2 in AD. Although MCL1 and BCL2 have largely redundant pro-survival functions, MCL1 has a unique regulatory mechanism governing its activity and biological functions, as discussed above. Therefore, MCL1's physiological function in AD must be further explored in detail by using *MCL1*-knockout AD animal models and clinical samples from patients with AD.

3.4 MCL1-dependent regulation of autophagy/mitophagy

A neuronal cell is compartmentalized into three main parts: the axon, dendrite, and cell body. Axonal dysfunction is closely correlated with various neurodegenerative disorders, and axonal degeneration is a pathological hallmark and primary cause of such diseases [111]. Axons actively grow to lengths as long as 1 meter in humans, and transmit chemical and electrical information over long distances, in a process with high nutrition and energy demands. Therefore, axons rely on autophagy to accomplish outgrowth over vast distances and the transmission of large amounts of information over their lifetimes. Consequently, axonal autophagy is closely correlated with axonal degeneration [112–114]. By introducing injury-induced axonal degeneration, elevated GSK3 β activity induces the phosphorylation of MCL1 at Ser140, thus leading to the dissociation of MCL1 from Beclin1, which in turn promotes proteasomal degradation of MCL1, thereby promoting axonal autophagy [115] (Figure 3). The elevated autophagy induces ATP production in axons and consequently leads to axonal regeneration. The phosphorylation-dependent MCL1 degradation in the mitochondria is mediated by FBW7. In agreement with FBW7-directed MCL1 ubiquitination, depletion of *Fbw7* suppresses axonal autophagy and subsequent axonal degeneration. Moreover, MCL1 negatively regulates axonal autophagy by inhibiting Beclin1, an autophagy receptor and component of the VPS34 complex responsible for phagophore nucleation [115, 116]. Like other BCL2 family proteins, MCL1 forms a complex with Beclin1 through the BH3 motif for its inhibition [117–119], that is required for suppressing

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autophagy [120] (Figure 4A). Beclin1 levels are down-regulated in vulnerable neurons in AD, and heterozygous *Beclin1*-knockout mice display AD-like phenotypes [27]. These observations suggest that proteasomal MCL1 degradation induces autophagy through alleviating the suppression of Beclin1, whereas *Beclin1* depletion or simultaneous BAK1 activation results in a shift from autophagy to apoptosis [117, 121]. Because the signaling pathways causing axonal degeneration in AD remain to be identified, further analyses on MCL1-associated signaling may aid in providing clues for identifying the underlying molecular mechanisms.

MCL1 is also involved in mitophagy. MCL1 and the pro-survival BCL2 family proteins BCL-XL and BCL-W suppress mitophagy induction by inhibiting Parkin recruitment to depolarized mitochondria [122] (Figure 4A). The pro-survival, anti-mitophagy effects of BCL2 family members probably occur through their direct binding to Parkin. In agreement with this possibility, BH3-only

proteins and BH3 mimetics antagonize this suppression [122]. Furthermore, in HeLa cells in which Parkin protein is absent, ectopic expression of MCL1 exerts anti-mitophagy activity through antagonizing the function of a mitophagy receptor, the activating molecule in Beclin1-regulated autophagy (AMBRA1), in mitochondria under steady-state conditions. AMBRA1 is a positive regulator of Beclin1, and its ablation leads to defects in neural-tube formation during development, owing to impaired autophagy [123]. Under normal conditions, MCL1 suppresses AMBRA1-mediated mitophagy. After mitophagy induction, increased AMBRA1 expression results in the translocation of the E3 ligase MULE from the cytoplasm to the mitochondria, where it triggers OMM protein ubiquitination and subsequent mitophagy induction. MULE simultaneously promotes MCL1 ubiquitination and degradation in a GSK3-phosphorylation-dependent manner, thus enhancing AMBRA1-mediated mitophagy [124].

Figure 4 | MCL1-mediated positive and negative regulation of mitophagy.

(A) MCL1 negatively regulates mitophagy through inhibiting mitophagy-activating factors, such as Parkin and Beclin1. (B) UMI-77 treatment facilitates mitophagy in an MCL1-dependent manner. MCL1 interacts with LC3 through its LC3-interacting region (LIR); therefore, MCL1 may act as a mitophagy receptor in this scenario. UMI-77 enhances binding between MCL1 and LC3, thus leading to mitophagy activation.

3.5 Targeting of MCL1 with BH3 mimetics in AD

MCL1 is a critical survival factor that has been a major focus in cancer research. Strategies are based on the mechanism of antagonism of pro-survival BCL2 family proteins by BH3-only pro-apoptotic proteins. Multiple cytotoxic stresses induce the expression of pro-apoptotic BH3-only proteins, which bind and antagonize pro-survival BCL2 proteins, thus leading to the liberation of the pro-apoptotic effector proteins BAK and BAX from the pro-survival BCL2 family proteins. Subsequently, release of cytochrome c from the space of the IMM is induced, and apoptosis ensues (Figure 2). The BH3-mimetic anti-BCL2 drug venetoclax, which has high affinity for BCL2, BCL-XL, and BCL-W, has been approved by the Food and Drug Administration and is clinically in use; however, this compound has no inhibitory effect on MCL-1, owing to the unique structure of the MCL1, which hampers the access of BH3-mimetic [125]. In contrast, several MCL1-targeting BH3-mimetic inhibitors have entered clinical trials [126].

Besides the pro-apoptotic action, BH3 mimetics induce dissociation between Beclin1 and pro-survival BCL2

family proteins, and consequently facilitate autophagy/mitophagy [119, 127] (Figure 5). MCL1 depletion, degradation, or inhibitory phosphorylation potentially lead to both autophagy and apoptosis, depending on the balance of Beclin1 and BAK/BAX levels. Furthermore, as described above, MCL1 suppresses mitophagy by inhibiting the recruitment of Parkin to depolarized mitochondria, whereas treatment with BH3 mimetics reverses this suppressive effect [122] (Figure 5).

A recent study has shown that targeting of MCL1 by the BH3 compound UMI-77 at a sub-lethal level induces autophagy without affecting mitochondrial dysfunction and apoptosis [54] (Figures 4B and 5). This study has further demonstrated that MCL1 interacts with LC3. This paradigm-shifting finding indicates a unique mechanism suggesting that MCL1 has an autophagy receptor function. Mechanistically, UMI-77 presumably exposes the LIR motif in MCL1 protein to LC3, thus facilitating the interaction of LC3 and MCL1 [128]. In agreement with this model, administration of UMI-77 alleviates AD phenotypes, such as by improving cognitive impairment, decreasing A β deposition in the brain, and diminishing

Figure 5 | MCL1-mediated regulation of autophagy and mitophagy in Alzheimer's disease.

(A) MCL1 interacts with and antagonizes the function of the autophagy-promoting factor Beclin1 and the apoptosis-promoting factors BAK/BAX through their BH3 motif. (B) Cytotoxic stresses that cause mitochondrial depolarization induce MCL1 post-translational modifications, including phosphorylation and ubiquitination, thereby resulting in proteasomal degradation of MCL1. Rapid removal of MCL1 releases Beclin1, thus leading to autophagosome formation and subsequent autophagy/mitophagy activation. (C) Therapeutic potential of MCL1 signaling in managing Alzheimer's disease. Targeting MCL1 in combination with administration of anti-apoptosis drugs may be a beneficial AD intervention. Distinctly from the canonical action of the pro-survival BCL2 family inhibitors, the BH3 mimetic UMI-77 induces autophagy.

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inflammatory-cytokine levels, in an APP/PS1-transgenic mouse model [54]. Given the relatively lower cytotoxicity of the UMI-77 treatment, this study has proposed an effective therapeutic intervention of AD without the undesirable effects of damaging mitochondrial function and inducing apoptosis.

4. CONCLUSION

This review focused on summarizing the possible molecular links between AD and MCL1 function in regulating autophagy/mitophagy and neuronal cell death. Mitochondrial dysfunction is a characteristic hallmark of AD, which is also considered a possible upstream event promoting A β and pTau pathology. Hence, restoring mitochondrial integrity and function may be a promising potential therapeutic approach for intervention. Cytotoxic stresses causing mitochondrial dysfunction induce rapid MCL1 degradation, thus providing a mechanism for cells to activate autophagy/mitophagy. Furthermore, effective autophagic induction has positive effects in AD, such as neuroprotection or resorting neuronal function. Therefore, promoting MCL1 degradation or inhibiting MCL1 activity that promotes autophagy/mitophagy may be a potential approach for treating AD. However, because suppressing MCL1 may cause cell death in neurons expressing the pro-apoptotic effectors BAK/BAX, MCL1 intervention may require combination treatments to inhibit the intrinsic apoptosis pathway (Figure 5). The recent finding of the beneficial effects of the BH3 mimetic UMI-77 may alleviate this concern, because UMI-77 treatment induces mitophagy without causing substantial mitochondrial toxicity or apoptosis induction in an AD mouse model (Figure 5). This study reveals an unexpected drug mechanism of action, in that the sub-lethal dose of the compound induces MCL1's unique pro-autophagic function. In conclusion, MCL1 is a potential therapeutic target; therefore, elucidating the molecular mechanisms involved in MCL1 signaling in mitochondria is expected to provide insight into a viable avenue of therapeutic intervention for AD.

DATA COLLECTION

The data cited in this review were retrieved from PubMed without limitations on the years of publication. For searching references, we used the keywords such as "Alzheimer's disease," or "Mitochondria," or "MCL1," or "Autophagy," or "Mitophagy".

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DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests.

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