PROTAC technology for the treatment of Alzheimer's disease: advances and perspectives

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ABSTRACT

Neurodegenerative diseases are characterized by the progression of neuronal degeneration, resulting in dysfunction of cognition and mobility. Many neurodegenerative diseases are due to proteinopathies arising from unusual protein accumulation and aggregation. The aggregation of misfolded proteins, such as β -amyloid, α -synuclein, tau, and polyglutamates, is a hallmark of Alzheimer's disease (AD). These aggregated proteins are undruggable targets and usually do not respond to conventional small-molecule agents. Therefore, developing novel technologies and strategies for decreasing the levels of protein aggregates is critical for the treatment of AD. Recently, the emerging proteolysis targeting chimera (PROTAC) technology has been considered for artificial, selective degradation of aberrant target proteins. These engineered bifunctional molecules engage target proteins, which are then degraded either by the cellular degradation machinery via the ubiquitin-proteasome system or through the autophagy-lysosome degradation pathway. Although PROTAC technology is preferable to oligonucleotides and antibodies for the treatment of neurodegenerative diseases, many limitations, such as their pharmacokinetic properties, tissue distribution, and cell permeability, must be addressed. Herein, we review recent advances in PROTAC technology, as well as PROTACs' limitations, for the pharmaceutical targeting of aberrant proteins involved in AD. We also review the therapeutic potential of dysregulated signaling, such as the PI3K/AKT/mTOR axis, for the management of AD.

Keywords: PROTAC, ubiquitin-proteasome system, protein degradation, autophagy, alzheimer's disease

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the leading cause of dementia in older people and the sixth leading cause of death [1]. In 2020, 6.2 million Americans 65 years of age or older were living with AD [1]. Early-onset AD due to inherited genetic mutations, such as those in APP, APOE4, and PS1, has also been reported among people under the age of 65 and affects approximately 200,000 Americans [2]. The earliest symptom is remembering new events, whereas more advanced symptoms include linguistic difficulties, mood swings, confusion, lack of motivation, self-neglect, and behavioral problems [3]. The pathogenesis of AD is not fully understood, and importantly is irreversible in late stages, such as those involving brain atrophy; therefore early AD diagnosis and treatment remain unmet needs.

Two pathological, aberrant structures in damaged neuronal cells have been identified as the main pathological hallmarks of AD: senile plaques and neurofibrillary tangles (NFT) [4]. Senile plaques are usually caused by the aberrant deposition of aggregated protein fragments called β -amyloid ($A\beta$; specifically $A\beta$ 42 and $A\beta$ 40) among nerve cells [5]. In this event, called amyloidosis of the brain, $A\beta$ peptides are cleaved from the amyloid precursor protein (APP) and aggregate as soluble toxic oligomeric $A\beta$. The aggregation of these soluble toxic oligomers results in the creation of hydrophobic surfaces; subsequently, insoluble fibrils are formed for disruption of the phospholipid bilayer [6], which is considered the main underlying cause of AD [7]. NFT are constructed from abnormal fibers of hyperphosphorylated tau protein inside neuronal cells [8]. Notably, hyperphosphorylation of tau results in the formation of tangles that eventually damage

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the structure and function of neuronal cells, and are considered another major pathological feature of AD [9]. In fact, the mechanistic principle underlying the formation of both senile plaques and NFT has been well established and is considered a key hallmark of AD [10, 11] (Figure 1).

2. PROTEIN DEGRADATION MACHINERY IN ALZHEIMER'S DISEASE

Normal cells undergo a self-controlling processes enabling homeostasis by preventing prolonged damage in response to environmental conditions. Several

Figure 1 | Therapeutic targets in Alzheimer's disease signaling.

The formation of NFT and senile plaques in AD signaling introduces several therapeutic targets. Four genes are mainly involved in AD pathogenesis: amyloid precursor protein (APP), apolipoprotein E (ApoE), presenilin 1 (PSEN1), and presenilin2 (PSEN2). Increased levels of A β peptides are caused by mutations in APP and PSEN, thus leading to the formation of A β 42, the main component of senile plaques. Cleavage of APP by either alpha-secretase or beta-secretase initiates extracellular release of soluble APP peptides, sAPP α , and sAPP β , and retains the corresponding membrane-anchored C-terminal fragments, C83 and C99. Alternatively, PSEN1/Nicastrin (NCSTN)-mediated gamma-secretase processing of C99 releases the A β proteins A β 40/42. In neuronal cell bodies, neurite outgrowth is stimulated by ApoE-containing lipoprotein lipase (LPL) *via* binding interactions between LRP and APP, thus resulting in the production of proteolytic fragments (A β) [161]. The accumulation of A β results in blocked ion channels, mitochondrial oxidative stress, activation of TNFR-regulated caspase 8, and ultimately neuronal cell death. However, GSK-3 phosphorylates tau at several sites, thus resulting in partial inhibition of tau's biological activity in AD [162]. Under abnormal Ca $^{2+}$ homeostasis, the stimulation of calpain mediates the cleavage of p35 to p25, thus activating CDK5 and leading to tau hyperphosphorylation and APP truncation [163]. Finally, the elevated Ca $^{2+}$ stimulates neuronal NO synthase, thereby leading to the production of nitrogen species and reactive oxygen species [164]. SNCA: α -synuclein; PEN2: presenilin enhancer (gamma-secretase subunit); APH1A: Aph-1 homolog a (gamma-secretase subunit); TNFR: tumor necrosis factor receptors; FADD: Fas-associated protein with death domain; CASP8: caspase 8; nNOS: neuronal nitric oxide synthase.

neurodegenerative diseases are caused by misfolded proteins that aggregate into β -sheet structures [6]. Cells are usually equipped with defense machinery against misfolded and aggregated proteins, which allows them to preserve homeostasis through two main strategies: (a) misfolded proteins are refolded with a plethora of molecular chaperones, and (b) if refolding is not possible, cells eliminate the aggregated proteins and consequently avoid neurodegenerative diseases. Loss of these defensive machineries increases the deposition of protein aggregates and results in the development of neurodegenerative diseases. Below, we introduce the ubiquitin-proteasome system (UPS) and autophagy pathways, which are the main defensive machineries for protein quality control in neuronal cells.

2.1 The ubiquitin-proteasome system

The UPS is essential for protein homeostasis through quality control in cells [12]. Ubiquitination results in the degradation of “unwanted” proteins via the 26S proteasome. In this process, ubiquitin, a 76-amino-acid protein,

is used for monoubiquitylation and/or polyubiquitination through its covalent attachment to a target protein. Ubiquitination occurs through a multistep, reversible enzymatic cascade including E1, E2, and E3 enzymes [13]. The eight amino group of N-terminus or Lysine residue (K6, K11, K27, K29, K33, K48, K63, and M1) of the ubiquitin protein provide different “ubiquitination” signals [14] with diverse functional consequences (Figure 2).

Protein turnover is essential for synaptic plasticity and memory in the nervous system, and should be considered in the regulation of protein stability and function in neuronal cells [15]. The UPS controls most protein functions in the postsynaptic response in neuronal cells. Meanwhile, protein aggregates are largely due to a decrease in degradation rather than an increase in synthesis [16]. Therefore, neurons fail to clear abnormal proteins in neurodegenerative proteinopathies. Understanding the UPS in each neuron is necessary for developing novel therapeutic approaches by enhancing proteasomal degradation for removing pathogenic aggregates in neuronal cells.

Figure 2 | Schematic representation of E3 ligase biology.

The ubiquitination of cellular proteins is triggered by the E1 enzyme, which utilizes the formation of Ub-AMP. After this catalytic reaction, ubiquitin is transferred to an E2 enzyme, and the thioester-linked E2-Ub complex is activated. Finally, an E3 ligase enzyme transfers the ubiquitinated protein from the E2 enzyme to a target protein. The ubiquitination process can be reversed by deubiquitinating enzymes (DUBs), which catalyze the cleavage of ubiquitin from target proteins or substrates. E1, ubiquitin-activating enzymes; E2, ubiquitin-conjugating enzymes; E3, ubiquitin ligases; Ub, ubiquitin.

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2.2 Autophagy

Autophagy, another important system, is a conserved degradation process supporting protein homeostasis in eukaryotic cells. Autophagy mainly processes larger cytosolic structures such as cellular protein aggregates and organelles within lysosomes [17]. The best-known form of autophagy is macro-autophagy, in which protein targets are sequestered in the phagophore, a cytosolic membrane compartment [18]. Mechanistically, cellular targets are engulfed by autophagosomes, which are transported *via* the cytoskeleton and subsequently fuse with lysosomes. This mechanism expedites the degradation of cytoplasmic substrates such as misfolded proteins (aggrephagy), highly loaded peroxisomes (perophagy), abnormal mitochondria (mitophagy), and pathogenic

organisms (xenophagy) [19]. In fact, several intracellular proteins, known as autophagy-related proteins, govern autophagy processes [20] (Figure 3).

Regarding the protective function of autophagy in neuronal physiology, the accumulation of abnormal tau proteins might be due to impaired autophagy within neurons [21]. Greater activation of autophagy increases degradation of the tau protein and decreases intracellular tau aggregation [22]. In addition, increased activity of autophagy effectively decreases the A β content, particularly in early stages of A β accumulation [23, 24]. Concordantly, A β has been found to arise from amyloid precursor protein within autophagosomes, thus indicating a unique link between the autophagy pathway and the formation of A β plaques [25].

Figure 3 | Protein degradation pathways in neuronal cells along with targeted protein degradation strategies.

PROTAC, proteolysis-targeting chimera; AUTAC, autophagy-targeting chimera; ATTEC, autophagosome-tethering compound; LYTAC, lysosome-targeting chimera; CI-M6PR, cation-independent mannose 6-phosphate receptor; LC3, microtubule-associated proteins 1A/1B light chain 3B; Ub, ubiquitin; E3, ubiquitin ligase; POI, protein of interest.

3. CHEMICALLY MEDIATED TARGETED PROTEIN DEGRADATION

Strategies of targeted protein degradation have recently emerged as modalities in drug discovery, wherein bifunctional small molecules hijack the cellular degradation machinery and direct protein targets for ubiquitin-mediated degradation. In 2001, the Crews group developed the first peptide-based bifunctional molecules, including the ligands of a target protein and a ubiquitin E3, and used them to construct proteolysis-targeting chimeras (PROTACs). This technology has since shown promise for the regulation of key target proteins that are otherwise untargetable. Several methods based on chemically mediated targeted protein degradation have been established, including hydrophobic tagging, molecular glues, autophagy-targeting chimeras, autophagosome-tethering compounds (ATTECs), lysosome-targeting chimeras (LYTACs), and PROTACs (Figure 3).

In the hydrophobic-tagging experimental system, the addition of hydrophobic tags (e.g., Boc₃Arg or adamantyl) to ligands induces structural changes and the formation of hydrophobic patches that initiate the unfolding of protein targets, which are subsequently degraded via the protein-homeostasis machinery [26]. In the molecular-glue strategy, degraders such as lenalidomide interact with the target protein and hijack a specific ubiquitin ligase, thus resulting in initiation of ubiquitination and subsequent target-protein degradation by the 26S proteasome [27].

Moreover, Takahashi *et al.* have recently developed a novel targeted-clearance strategy termed autophagy-targeting chimeras (AUTACs), which are bifunctional molecules conjugated by small molecules that induce autophagy [28]. In brief, S-guanylation (with guanine derivatives) is used to tag chimeric molecules (including the guanine component and a specific ligand for a desired protein target) to selectively direct protein substrates to the autophagy system for programmed destruction. AUTACs result in the removal of fragmented mitochondria and the biogenesis of normal mitochondria. Thus, AUTACs might provide a modality for developing autophagy-based drugs with specific targets to combat AD. In addition, other strategies using autophagy for targeted protein degradation have been reported, including ATTECs and LYTACs [29]. In brief, ATTEC molecules bind the target protein along with LC3, protein that recruits target proteins to autophagosomes for degradation via autophagy. LYTACs are heterobifunctional molecules that target extracellular and membrane-associated proteins by conjugation, and engage endosomes and lysosomes through the recruitment of protein targets to the lysosome-shuttling receptor at the cell surface [29].

4. PROTACS

PROTACs are heterobifunctional molecules that degrade target proteins by hijacking the powerful

cellular degradation systems [30–32]. PROTACs recruit target proteins to an E3 ubiquitin ligase (usually MDM2, Von Hippel Lindau (VHL), IAPs, and CRBN ligases) via an optimal linker (Figure 4). The formation of a complex between the protein of interest (POI) and the E3 ligases triggers POI ubiquitination and subsequent degradation by the 26S proteasome in eukaryotic cells [33–38] (Figure 5). The first PROTAC degrader was developed to target the androgen receptor for the treatment of metastatic castration-resistant prostate cancer [39, 40]. After validation of the proof of concept, the use and development of PROTAC technology markedly increased in industrial and academic settings. This broad adoption is mainly because PROTACs: 1) can block both enzymatic and non-enzymatic functions of proteins, 2) can destroy “undruggable” POIs, 3) have high specificity and selectivity, 4) can rapidly and reversibly eliminate protein targets, and 5) have promise in overcoming drug resistance [41]. Drug resistance is a major problem in anticancer therapy, and PROTAC technology is expected to generate more effective drugs to circumvent drug resistance. The use of PROTACs should be highly beneficial because of its novel mechanism of action for decreasing target-protein levels with lower drug doses than conventionally used. The first oral PROTACs assessed in clinical trials were ARV-110 for prostate cancer and ARV-471 for breast cancer, both from Arvinas [42]. In brief, PROTAC molecules can be used to cure autoimmune and inflammatory conditions, and treat diseases such as allergies, asthma, cardiovascular disease, and AD [43].

5. PROTEINS TARGETING PROTACS IN THE MANAGEMENT OF ALZHEIMER'S DISEASE

Here, we introduce several PROTACs developed for the elimination of misfolded proteins, which are a major cause of neurodegenerative diseases. PROTACs provide excellent therapeutic benefits in targeting pathogenic proteins for degradation. We introduce the major dysregulated proteins, including tau and α -synuclein (α Syn), that cause neurodegenerative diseases such as AD. We will discuss the major aberrant protein/signaling pathways, contributing to AD, including the hypoxia/HIF1 α , phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/AKT, glycogen synthase kinase 3 β (GSK-3 β), and bromodomain and extra-terminal domain (BET) pathways.

5.1 The main pathological targets in Alzheimer's disease

5.1.1 Tau. The tau proteins are abundant in neuronal cells, where they stabilize microtubules in axons; consequently, they are known as microtubule-associated proteins for axonal transport [44–48]. Increased aggregation of tau protein is correlated with synaptic dysfunction, thus leading to abnormal localization of tau from axons to the somatodendritic region. NFT are aggregations of tau protein in neuronal cells in AD [44]. Hyperphosphorylated tau proteins aggregate and form NFT. Tauopathies,

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Figure 4 | E3 ligase ligands commonly used for PROTACs.

Derivatives of thalidomide targeting Cereblon (CRBN) and the ligand of Von Hippel Lindau (VHL) are shown. The asterisk shows the point of attachment to the linker.

characterized by the development of neurofibrils from the hyperphosphorylation of tau, can arise in atypical Parkinson syndromes [49]. Dysregulation of tau is also an important issue in frontotemporal dementia and in A β toxicity. Thus, tau has been suggested as an attractive target for the potential treatment of AD.

In 2016 and 2018, Chu and Lu *et al.* reported that peptide forms of PROTACs can degrade tau proteins [50, 51]. Interestingly, the peptidic PROTAC TH006 degrades tau in the CA3 region of the hippocampus *in vivo* (Table 1). Later, in 2019, Silva *et al.* constructed a set of unique

PROTACs by using a tau PET tracer as a warhead [52] for targeting tau in human differentiated frontotemporal dementia (FTD) neurons. PROTAC T807 treatment sufficiently degraded the wild-type (WT) protein and the tau variants A152T and P301L in neurons, with K_d values of 1.8, 2.1, and 1.7 μ M, respectively. The authors also established a second PROTAC, QC-01-175, that specifically degrades tau variants in neurons with FTD rather than healthy neurons (Figure 6 and Table 1). In addition, QC-01-175 rescues stress vulnerability in patient-derived neuronal cell models of FTD.

A. Natural degradation through the ubiquitin-proteasome system (UPS)

B. PROTAC-induced degradation of POI through UPS

Figure 5 | Schematic illustration of PROTAC mechanisms of action.

(A) The E1 Ubiquitin-activating enzyme initiates transfer of Ubiquitin (Ub) to a target protein through the E1-E2-E3 enzymatic cascade. Then, the ubiquitinated target protein is degraded by the 26S proteasome. (B) Without PROTACs, the target protein is not recruited for ubiquitination. The PROTAC molecule attaches to a POI and the E3 ubiquitin ligase, thus resulting in ubiquitination and subsequent proteasomal degradation.

In 2021, Wang *et al.* developed a PROTAC using a linker connecting tau to a VHL ligand [53] (Figure 6). In *in vitro* and *in vivo* experiments, their PROTAC, C004019, cleared tau protein under both physiological and pathological conditions. Interestingly, a single dose or infrequent administration of the PROTAC (once per 6 days) resulted in a sustained tau decrease and alleviated A β -induced neurotoxicity in the brain in a 3 $\times$ Tg-AD mouse model, without causing clear abnormalities. Robust tau clearance in the hippocampus and cortex in mice was observed, along with improvements in synaptic and cognitive function. These findings indicate that this PROTAC is an efficient drug candidate for tauopathies and the treatment of AD (Table 1).

5.1.2 α -Synuclein. The amino acid sequence of α Syn includes three distinct domains: the N terminus, the C terminus, and a central hydrophobic region through which α -synuclein assembles into amyloid fibrils [54]. Misfolded and aggregated α Syn expands in a prion-like fashion among cells, thus resulting in fibril amplification and progression to synucleopathies [55]. Because the deposition of α Syn is initiated in the enteric nervous system, the pathogenesis of synucleopathies is assumed to begin in enteric nerves before deposition in the brain.

In fact, patients with Parkinson's disease (PD) experience gastrointestinal problems before motor deficits [56]. The symptoms of PD and AD are moderately similar, but AD pathologically affects the cerebral cortex and hippocampus, whereas PD occurs mainly in the substantia nigra [57]. Although patients with AD show PD symptoms, and *vice versa*, patients with PD show clearer cognitive dysfunction; these findings suggest a pathological synergy between A β and α Syn. Studies have revealed that α Syn is highly expressed in brain regions with abundant AD lesions, and the enrichment of α Syn in the cortical region is correlated with the presence of A β [58].

Recently, Kargbo *et al.* have developed a bifunctional PROTAC compound that targets α Syn protein [59]. Their compound was developed by using a VHL moiety to target POIs. The linker places the VHL E3 ubiquitin ligase in proximity to target proteins for UPS degradation. The representative PROTAC has been found to prevent the accumulation and aberrant aggregation of α Syn protein in HEK293 cells stably expressing TREX α Syn A53T [59].

5.2 Aberrant target proteins dysregulated in the AD microenvironment

5.2.1 Hypoxia/HIF1 α signaling. Insufficient amounts of oxygen, a condition known as hypoxia, affect the

Table 1 | Properties of PROTACs with peptide-mediated or chemical-mediated targeting

PROTAC	Entity ^c	Tau recognized	Linker	Recruiting E3 ligase ^a	Cell- penetrating peptide	IC ₅₀ (μM)	K _d value (μM)	In vivo study	PK study	PMID
Peptide-mediated targeting	TH006	YQQYQDATADEQG	GSGS	ALAPVIP (VHL)	RRRRRRRR ^a	None	0.3944 ± 0.1589	Tau degradation in the CA ₃ region of the hippocampus	Not reported	27105281
	Peptide 1	YQQYQDATADEQG	GSGS	LDPETGEYL (Keap1)	RRRRRRRR	None	Not reported	Not reported	Not reported	29407955
Chemical-mediated targeting	QC-01-175	WT ^b		CRL4 ^{CRBN}		8.559	1.2 ± 0.44	Not reported	Not reported	30907729
		A152T ^b					1.7 ± 0.54			
		P301L ^b					2.5 ± 1.31			
	T807	WT		CRL4 ^{CRBN}		0.144	1.8 ± 0.99	Not reported	Not reported	30907729
		A152T					2.1 ± 0.50			
		P301L					1.7 ± 0.77			
	C004019	Total, pS214, and pS404 tau		VHL		0.00785	Not reported	Robust tau clearance in the hippocampus and cortex; improvement in synaptic and cognitive function	Tmax (h) = 0167; Cmax (ng/ml) = 10.8; AUClast (h×ng/ml) = 8.42	33859747

^aRepresents poly-D-arginine.^bRepresents human recombinant biotinylated-tau WT, A152T, and P301L, respectively.^cThe name and identification of drugs.

Tmax: time point at which the drug concentration was highest; Cmax: maximum drug concentration; AUClast: area under the curve (the integral from the beginning to the last point in time).

Figure 6 | Chemical structure of tau-targeting PROTACs: QC-01-175 and C004019.

pathological and physiological function of cells [60]. Under hypoxia, prolyl hydroxylases, major molecular sensors of oxygen, cannot catalyze prolyl hydroxylation of hypoxia-induced factor alpha (HIF1 α) and AKT [61, 62]. Hydroxylated HIF1 α is recognized and poly-ubiquitinated by the E3 ligase VHL, thus resulting in HIF1 α degradation by the UPS [63, 64]. Hypoxia and/or HIF1 α accumulation in VHL-deficient cells results in the recruitment of HIF1 β and the transcriptional regulation of numerous genes [64]. Phosphorylation of histone H2AX plays an important role in the maintenance and inhibition of HIF1 α degradation under hypoxia [65, 66].

Decreased oxygen in the brain is also associated with neurodegenerative diseases such as AD. In fact, the central nervous system (CNS) is highly sensitive to the oxygen supply, such as during aging, in which delivery of oxygen via the cardiovascular system is diminished [67]. Hypoxia also supports the formation of plaques and consequently leads to memory deficits in a mouse model for AD [68]. In the brain under hypoxia-driven AD, several molecular signaling pathways are also activated, thereby supporting oxidative stress [69], AKT/mTOR activation [70], angiogenesis [71], and metabolic activation [72]. Hypoxia rapidly induces the expression of A β , cyclooxygenase-2 (COX-2), and presenilin 1 (PS1), thereby resulting in neuroinflammation in the brain in AD [73]. However, under extended hypoxic conditions, Ca²⁺ channels are upregulated, thus supporting the production of A β [74]. Importantly, HIF1 α triggers transcriptional expression of VEGF [75] and BACE1 (a major biomarker of AD) [76], and consequently increases A β deposition and neurotic plaque production in a transgenic mouse model [76]. HIF1 α therefore induces tau phosphorylation partly through the regulation of several signaling pathways, including the GSK3 β , cyclin-dependent kinase 5 (CDK5), and mTOR pathways [77, 78]. Moreover, Glut1 and Glut3, other HIF1 α -target genes and major brain glucose transporters, show diminished expression in AD [79].

In early 2004, Schneekloth *et al.* reported that peptide-based PROTACs recognize the transcription of HIF1 α and bind VHL [80]. The authors added a poly D-arginine tag to the peptide sequence to facilitate cell penetration. Interestingly, the discovery of a small molecule inhibiting the interaction between HIF1 α and VHL led to the establishment of several PROTACs linking VHL to other target POIs with high specificity and affinity [81, 82]. Aging can induce hypoxia/HIF1 α , thus resulting in the dysregulation of several molecular signaling pathways in neurons and subsequent AD pathogenesis; these aspects should be considered for careful selective degradation with novel PROTAC technology.

5.2.2 PI3K/mTOR signaling. PI3K, a member of the PI3K/AKT/mTOR signaling pathway, functions as a phosphatidylinositol kinase. It primarily regulates apoptosis, proliferation, and differentiation of cells, and its overexpression drives tumorigenesis. Moreover, mTOR responds to a wide range of extracellular stimuli in the regulation of cell growth and metabolic homeostasis [83]. The mTOR pathway also regulates several diseases, including cancer [84], diabetes [85], and neurodegenerative pathological conditions [86]. In fact, inhibiting mTOR increases the lifespan of several organisms, such as *C. elegans* [87] and mice [88], whereas rapamycin, an mTOR-specific inhibitor, extends their lifespan [89–93]. Importantly, mTOR kinase promotes tau phosphorylation by regulating multiple kinases, including GSK3, protein kinase A, and CDK5 [94]. Moreover, mTOR directly phosphorylates and inhibits protein phosphatase 2A, a major phosphatase downregulated in the AD brain [95], thus increasing tau phosphorylation [96]. Furthermore, downstream targets of mTOR, such as S6K and eukaryotic translation factor 4E, increase the mRNA translation of Tau, thereby indicating that overactivation of mTOR results in accumulation of tau protein [97]. Unexpectedly, the administration of A β in the hippocampus in normal mice has been found to activate the

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mTOR pathway; these findings demonstrate the role of amyloid precursor in the activity of mTOR signaling [98]. Furthermore, rapamycin decreases cognitive deficits in tau pathology through amelioration of A β in the brains of mice with AD [99]. Thus, mTOR disrupts tau homeostasis and results in the aggregation of tau and the formation of NFT in AD pathogenesis. These findings suggest a potential strategy for the treatment of AD through targeting mTOR.

Recently, several PI3K inhibitors have been established, most of which have limitations because of inadequate selectivity and adverse effects [100, 101]. Consequently, the improvement of unique PI3K-targeting PROTACs has been recognized as a powerful strategy. In 2018, Jiang *et al.* established a set of prospective PI3K degraders using lenalidomide in the link with ZSTK474 inhibitors [102]. Although this PROTAC revealed lower enzymatic activity than ZSTK474, it successfully degraded PI3K at 10 μ M, and decreased the phosphorylation of GSK-3 β , S6K, and AKT in the PI3K/AKT/mTOR signaling pathway.

5.2.3 AKT. AKT, a central member of the PI3K/AKT/mTOR signaling pathway, is a serine/threonine kinase that regulates several cellular processes such as survival, proliferation, and metabolism. Some gain-of-function mutations and/or the activation of oncogenes such as PI3K and receptor tyrosine kinases, and/or the loss of the tumor suppressor function of PTEN, can lead to hyper activation of AKT in the cancer progression [103]. Moreover, GSK3 β -mediated tau phosphorylation is critical for the initiation of AKT-sulfhydrylation [104]. Therefore, AKT is considered to be central to PI3K/AKT/mTOR signaling and should be an interesting therapeutic target to combat AD.

In 2019, You *et al.* developed a specific small-molecule degrader based on the conjugation of a CRBN ligand with GDC-0068 as an AKT inhibitor [105]. This engineered PROTAC has been found to inhibit AKT1, AKT2, and AKT3 with IC₅₀ values of 2.0 nM, 6.8 nM, and 3.5 nM, respectively, in contrast to GDC-0068's IC₅₀ values of 5 nM, 18 nM, and 8 nM, respectively. In addition, this PROTAC destabilizes all three isoforms and decreases AKT-downstream signaling. The engineered PROTAC also downregulates cell proliferation much more efficiently than its parental inhibitor and therefore should be valuable for targeted degradation of AKT.

5.2.4 GSK-3 β . GSK-3 is a serine/threonine protein kinase and a member of the phosphotransferase family [106]. Pathologically, GSK-3 β regulates several process through tau phosphorylation and also results in the production of A β peptide, thus causing NFT and amyloid plaques in AD [104]. Moreover, the proinflammatory function of GSK-3 β causes neuronal loss [107-109] and neurodegenerative disease [110]; therefore, this kinase should be considered therapeutic target for AD treatment [111-113]. In 2021, Jiang *et al.* reported the first set of GSK-3 β -targeting PROTACs, developed by using CRBN

as an E3 ubiquitin ligase [114]. Their PROTAC efficiently induces degradation of GSK-3 β protein (44%) with an IC₅₀ value of 2.8 μ M; this PROTAC should therefore be considered as an effective GSK-3 β degrader.

5.2.5 BET. Dysregulation of inflammation, a critical process in the pathology of many CNS diseases, occurs through several pathways, including NF- κ B and Nrf2 signaling in inflammation. BET proteins include four members (BRD2, BRD3, BRD4, and BRDT) that play critical roles in the transcriptional regulation of the inflammatory response [115, 116]. For example, BETs assemble the histone-acetylation-dependent chromatin complex for the expression of inflammatory genes. Early studies have also shown that BET inhibition activates anti-inflammatory pathways; therefore, BRD2, BRD3, and BRD4 proteins may play critical roles in AD and other neuroinflammatory disorders. [116-120]. Targeting BET proteins with a small-molecule inhibitor (JQ1) has been found to downregulate several proinflammatory regulators, such as IL-1 β and TNF- α , and to be followed by tau phosphorylation at Ser396 in the frontal cortex and the hippocampus in the 3xTg mouse model of AD [121]; however, these mice do not show memory deficits and amelioration of learning. In the APP/PS1 AD mouse model, JQ1 enhances long-term potentiation (LTP) and cognitive function [122]. Moreover, JQ1 activates the expression of hippocampal genes responsible for the activation of ion channels and DNA repair [122]. Chemical probes such as pan-BETi(s) serve as ideal PROTACs for targeting BET proteins, owing to their potential for BET identification and recruitment.

The first PROTAC-targeted BET proteins (dBET1) were identified in 2015 and included an E3 ligase and a BRD4 BD binding moiety (JQ1 or OTX015) [123]. The BET PROTACs have been shown to identify and recruit the CRBN E3 ubiquitin ligase for efficient and selective degradation of BRD4 protein *in vitro* and *in vivo* [123-127]. Moreover, a proteomic study has indicated that dBET1 decreases BET proteins by 8- to 10-fold, along with several other BET-target proteins [123].

6. THE STRENGTHS AND WEAKNESSES OF PROTAC TECHNOLOGY

6.1 Advantages

PROTAC technology is favorable for the treatment of CNS diseases because it has several advantages over traditional approaches [128]. The main advantage is the ability for targeted degradation of undruggable proteins in the CNS. PROTACs have higher specificity than other methods and can selectively degrade different isoforms of proteins expressed by the same gene. Furthermore, PROTACs bypass the potential toxic effects that can develop with pharmacological approaches. In fact, PROTACs can inactivate target-protein function without directly binding target proteins for long time periods. Moreover, PROTACs can be designed on the

basis of catalytic reactions and consequently can be reused for many cycles until the target proteins in cells are eliminated. Furthermore, because PROTACs have sub-stoichiometric catalytic activity [129], administration of very low concentrations is sufficient to degrade a target protein. Therefore, developing active drugs by using PROTACs is highly feasible, and the inhibition of target protein does not require high concentrations of drug [130, 131]. Overall, PROTACs may be a highly effective method to target and destroy undruggable proteins involved in the pathophysiology of many CNS disorders including AD.

6.2 Disadvantages

Several limitations may prevent the development of PROTAC drugs for clinical applications in the future. Because PROTAC technology uses drugs for dual targets, the constructed compounds would have a high molecular weight for being easily synthesized [30, 131]. Therefore, they cannot be simply dissolved for oral absorption because of transmural issues, thus posing a major pharmacokinetic barrier. For example, a major issue is blood-brain-barrier permeability, a limiting factor in many pharmacological approaches. As described above, the efficacy study of the PROTAC C004019 (section 5.1.1) [53] suggests that PROTACs might also effectively penetrate the blood-brain barrier [30].

Another possible limitation is localization of PROTACs to specific brain regions, as is desirable to combat neurodegenerative diseases such as AD. For successful use of PROTACs, the E3 ligase must be expressed in the target region. However, some E3 ligases (e.g., CRBN) [132] are differentially expressed across brain areas, thus potentially complicating disease treatment. For example, tau accumulation occurs progressively in the brain regions affected by AD, starting in the locus coeruleus and entorhinal cortex and ending in the primary visual cortex [133]. Therefore, depending on disease progression, PROTACs would need to precisely target tau in specific regions of the brain. Managing drugs for colocalization with and/or without expression of E3 ligase in the affected brain regions is challenging, and new technology is needed to solve this emerging concern [134]. Furthermore, many CNS disorders are associated with expansive decreases in proteasome catalytic activity [135]. Consequently, even if a PROTAC can ubiquitinate a POI, it still might not degrade the protein. Thus, decreased proteasome function remains a barrier to PROTAC-mediated degradation of target proteins divergently expressed in the brain for the treatment of AD.

7. DISCUSSION

PROTACs are a powerful and attractive strategy studied and developed in both academic and industrial settings. Recently, they have been widely investigated for the treatment of several diseases including cancer, neurodegeneration, immune disorders, cardiovascular

dysfunction, fatty liver disease, and viral infections. Here, we discuss AD-specific PROTACs and several others that have been used for targeting aberrant proteins/signaling pathways that are dysregulated in the tumor microenvironment. However, because of their similar structures and mechanisms, we introduce these molecules as potential PROTACs for AD treatment. We reviewed two peptidic PROTACs developed for targeting tau in the treatment of AD [50, 51]. Although they degrade tau, the subunit protein of one of the major hallmarks of AD, their application has been limited, partly because of their intrinsic proneness to protease degradation and their poor membrane permeability *in vivo*. The stability of those peptides might be improved by substitution of unnatural amino acids, backbone modifications, and cyclization [136], and their cell-membrane permeability could be modified by increasing their lipophilicity and decreasing their hydrogen bonding [137]. Silva *et al.* have also established another type of PROTAC using a tau PET tracer as a warhead; however, application of these PROTACs has not been validated *in vivo* [52]. In fact, the construction of PROTACs such as warheads with high affinity toward target proteins might be not sufficient for the generation of active PROTACs [138]. Therefore, steric-structure induction by the binding of target protein to hijacked E3 ligase facilitates the transfer of ubiquitin from E2 to the target protein and potentiates PROTAC activity. This ternary complex enables PROTACs to degrade large molecules, such as protein aggregates, which normally cannot pass into the proteasome's barrel-like structure for degradation [15]. Through this approach, Wang *et al.* have established the PROTAC C004019, which efficiently induces clearance of tau in the brain in hTau and 3xTg-AD transgenic mice. Thus, C004019 may potentially pass through the proteasome's barrel-like structure and cross the blood-brain barrier, unlike most large drugs. Although C004019 markedly improves synaptic and cognitive function in these mice, its selectivity for recognition of tau species (WT and phosphorylated tau) is poor, perhaps partly because of the structure of tau, which is a natively unfolded protein. Thus, new challenges exist to improve PROTAC capabilities.

Although aging is a major cause of cancer and AD [139], some studies have revealed that AD pathogenesis protects against cancer, and *vice versa*, although the underlying mechanisms remain elusive [140, 141]. Notably, cancer and AD pathologically share common features. For example, a high level of cell-cycle re-entry, which is required for cancer pathogenesis, has also been observed in patients with AD [142, 143], but instead of cell division, the neuronal cell cycle is aborted, and cell death occurs [144, 145]. The activation of CDK2, CDK4, CDK5, and caspases is significantly elevated during the cell cycle, and results in APP phosphorylation, APP proteolysis, and A β production [146-148]. The role of mTOR activation in the initiation of proliferation suggests its function in cell-cycle re-entry

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of neuronal cells and the pathogenesis of AD [149]. The activation of mTOR is also believed to drive neurodegeneration through tau activation [150], thereby leading to NFT accumulation. Moreover, inactivation of the mTOR pathway results in the activation of the activation of autophagy for clearance of A β from the cells [151]. In the brain tumor microenvironment, adenosine monophosphate protein kinase (AMPK) might be activated under energy stress and consequently result

in the activation of FOXO3 [152] and the protection of neuronal cells. This energy stress is due to the high metabolomic rate of cancer cell proliferation and the hijacking of energy in the brain microenvironment. Meanwhile, mTOR signaling and consequent cell-cycle re-entry are inhibited by AMPK [153], and FOXO increases the expression of antioxidant enzymes, thereby decreasing cell damage [154, 155] and inhibiting neurodegeneration. Together, mTOR-mediated cell growth and proliferation not only drive cancer cell progression but also cause neuronal cell arrest. Therefore, targeting the PI3K/mTOR/AKT axis provides an excellent therapeutic option for the management of AD and tumor microenvironments (Figure 7).

In section 5.2, we briefly introduced several PROTACs established to target HIF1 α , PI3K, AKT, and BET in the tumor microenvironment, with its aberrant signaling. Most of these PROTACs efficiently degrade their own target proteins, such as BET, with high sensitivity and selectivity. Therefore, the application of these PROTACs has great potential in targeting the AD microenvironment. Although cancer and AD pathologically share common features, their disease-causing mechanisms substantially differ. Given that most established PROTACs have been studied primarily in the cancer disease setting, their formulation and mechanisms of action should be carefully designed to combat AD. Currently, two PROTACs, ARV-110 and ARV-47, have been used in clinical trials for the treatment of prostate and breast cancer, respectively, by targeting of androgen receptor and estrogen receptor (from Arvinas). Both show acceptable safety, and ARV-471 has been found to be well tolerated at all tested dose levels without severe adverse effects. Interestingly, ARV-471 has a synergistic effect on tumor inhibition in combination treatment with kinase inhibitors such as CDK4/6 inhibitors. Therefore, the combination of PROTAC with chemotherapy, antibody therapy (in immunotherapy), and small-molecule inhibitors might provide an alternative approach for the treatment of diseases including cancer and AD.

Figure 7 | Therapeutic potential of the PI3K/AKT/mTOR axis for the management of AD and cancer.

Over long-term physiological stimulation, such as aging followed by mitochondrial dysfunction, oxidative stress and metabolic stress might accumulate and trigger PI3K/AKT/mTOR signaling, thus resulting in strong activation of cell-cycle re-entry in neuronal cells. In contrast to cancer, in AD, the neuronal cell cycle is aborted, and cells proceed to death instead of division. Moreover, a significant elevation in CDK2, CDK4, and CDK5, and activation of caspases during cell-cycle re-entry leads to APP phosphorylation, APP proteolysis, and A β production. Furthermore, activation of the mTOR pathway inactivates autophagy, the process required for the clearance of A β in neuronal cells. After cancer cell proliferation in the brain, thus hijacking energy from neurons, this energy stress stimulates AMPK and activates FOXO3, which protects neuronal cells in the brain tumor microenvironment. FOXO increases the expression of antioxidant enzymes, which decrease the damage to neuronal cells. Dashed arrows require additional investigation of the molecular network in the indicated condition.

8. CONCLUSION AND PERSPECTIVES

PROTAC technology enables the discovery of new therapeutic agents with the unique ability to degrade rather than inhibit “undruggable” proteins. They can be used to address several concerns associated with the use of traditional small molecules, which may have poor selectivity, and result in adverse effects and drug resistance. However, the toxicity of PROTACs may limit future drug development, partly because of the effects of off-target degradation. PROTACs completely degrade proteins via the UPS, whereas parental small molecules or compounds are required only to inhibit protein functions. Therefore, the combination of small molecules with a tissue-specific E3 ligase is highly advantageous for the selective degradation of target proteins. Moreover, the structure of PROTACs is generally complex, and their

synthesis is complicated because of their high molecular weight (800–1000 kDa). These aspects should be recognized for further modification and screening to improve brain membrane permeability. Further optimization of the structure and synthesis of PROTACs is necessary to provide a stable platform for drug discovery. To this end, the application of crystallography may aid in understanding of the structural mechanisms of PROTACs. For example, structural analyses of the VHL and/or Cereblon E3 ligase proteins have demonstrated the shape and position of the Cullin-RING E3 ubiquitin ligase along with E2 and target proteins in detail, thus enabling ubiquitin transfer to be targeted [156].

As described herein, confirming that all proteins of interest will be degraded by a PROTAC in the absence of ligands is impossible. Therefore, the selection of new ligands and validation of targets will be another focus in PROTAC development. More than 600 E3 ligases exist in the human genome, but only several have been used in PROTAC technology. Therefore, vast possibilities exist for expanding screening efforts to identify suitable E3 ligases. Furthermore, additional E3 ligases must be investigated to avoid off-target effects and rapid responses to brain diseases, particularly AD. Finally, recent advancements in CRISPR-dCas9 technology may be helpful to stimulate proteasome function in brain disorders with extensive decreases in proteasome catalytic activity [157]. Interestingly, IU1, a compound inhibiting ubiquitin-specific protease 14 (USP14), has been found to improve protein degradation *in vitro* [158] and in mouse brain tissue [159], although proteasome function is not broadly enhanced in the brain [160]. These findings suggest a viable avenue for careful design of combination therapies using PROTACs, monoclonal antibodies, RNA interferes, and/or small-molecule inhibitors to combat AD.

9. DATA RETRIEVAL

This review was accomplished through the use of electronic databases, such as PubMed, Scopus, and Web of Science, for finding articles associated with AD, aberrant signaling pathways, as well as the application of PROTACs. We used the keywords such as (“Alzheimer’s disease” OR “PROTAC” OR “ubiquitin proteasome system”, OR “therapeutic target”, OR “aberrant signaling”) (title/abstract/keywords). In general, the focus was on PROTACs affecting aberrant signaling pathways in AD. Data were gathered without date limitations until November 2021. The lists of references in the articles were also manually searched for aberrant signaling pathways recognized as crucial therapeutic targets in AD.

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DECLARATION OF COMPETING INTERESTS

The authors declare no conflicts of interest associated with this manuscript.

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