



Published in final edited form as:

Acta Mater Med. 2022 ; 1(1): 24–41. doi:10.15212/amm-2021-0001.

PROTACs technology for treatment of Alzheimer's disease: Advances and perspectives

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Abstract

Neurodegenerative diseases (NDs) are characteristic with progression of neuron degeneration, resulting in dysfunction of cognition and mobility. Many neurodegenerative diseases are because of proteinopathies that results from unusual protein accumulations and aggregations. The aggregation of misfolded proteins like β -amyloid, α -synuclein, tau, and polyglutamates are hallmarked in Alzheimer's disease (AD), which are undruggable targets, and usually do not respond to conventional small-molecule agents. Therefore, developing novel technology and strategy for reducing the levels of protein aggregates would be critical for treatment of AD. Recently, the emerging proteolysis targeting chimeras (PRPTACs) technology has been significantly considered for artificial and selective degradation of aberrant target proteins. These engineered bifunctional molecules engage target proteins to be degraded by either the cellular degradation machinery in the ubiquitin-proteasome system (UPS) or via the autophagy-lysosome degradation pathway. Although the application of PROTACs technology is preferable than oligonucleotide and antibodies for treatment of NDs, many limitations such as their pharmacokinetic properties, tissue distribution and cell permeabilities, still need to be corrected. Herein, we review the recent advances in PROTACs technology with their limitation for pharmaceutical targeting of aberrant proteins involved in Alzheimer's diseases. We also review therapeutic potential of dysregulated signaling such as 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 accountable for the leading case of dementia in elder people and ranks the sixth causes of death [1]. In 2020, it is estimated that 6.2 million American ages 65 and older are living with and suffering from Alzheimer's disease [1]. Early-onset AD has also been reported for patients under

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Declaration of Competing Interest

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

age of 65, which is approximately occurring in 200,000 Americans because of the inherited genetic mutations, such as APP, APOE4, PS1, *etc* [2]. The most early symptom is trouble in memorizing new events, but in advanced level the problems include with linguistic, mood swing, confusion, losing motivation, self neglects, and behavioral issues [3]. The pathogenesis of Alzheimer's disease is still not fully understood to date, and importantly its pathogenesis is irreversible at late stage, such as brain atrophy, thereby it requires unmet need for early diagnosis and treatment.

There are two pathological and aberrant structures in damaged neuronal cells that have been identified as main pathological hallmarks in AD, including senile plaques and neurofibrillary tangles (NFT) [4]. Usually senile plaques are caused by the aberrant deposition of aggregated protein fragments that is named beta-amyloid (A β 42 and A β 40) and constructed among nerve cells [5]. This event is called "amyloidosis of brain", where A β peptides are cleaved from the amyloid precursor protein (APP) and aggregated as oligomeric A β . These pathogenic proteins aggregate into soluble toxic oligomers for the creation of hydrophobic surfaces before formation of insoluble fibrils and disruption of the phospholipid bilayer [6], which are considered as the main reason of AD [7]. On the other hand, the neurofibrillary tangles are constructed from the abnormal fibers of the hyper-phosphorylated tau protein inside neuronal cells [8]. Notably, hyper-phosphorylation of tau constructs tangles that eventually damage the structure and function of neuronal cells, which is considered another main pathology in AD [9]. In fact, the mechanistic principal of both senile plaques and neurofibrillary tangles have been well established and regarded as key hallmarks of Alzheimer disease [10, 11] (Figure 1).

2. Protein Degradation Machinery in Alzheimer's disease (AD)

Normal cells are gained self-controlling quality process to achieve homeostasis through prevention of prolonged damage in response to environmental conditions. Several neurodegenerative diseases are caused by misfolded proteins that aggregated into a β -sheet structure [6]. Usually cells are equipped with a defense machinery against misfolded and aggregated proteins to preserve homeostasis through two main strategies: (a) they refold misfolded proteins using a plethora of molecular chaperones, and (b) if cannot refold the misfolded proteins, cells will eliminate the forms of aggregated proteins for relieving neurodegenerative diseases. Outcome of losing these defensive machinery systems increase the deposition of protein aggregates, subsequently develop neurodegenerative diseases. Below we introduce the ubiquitin proteasome system (UPS) and autophagy pathways, which are main defensive machinery systems for protein quality control in neuronal cells.

2.1. The Ubiquitin Proteasome system

The ubiquitin proteasome system (UPS) is essential for protein homeostasis for quality control in cells [12]. Ubiquitination activities degradation of "unwanted" proteins *via* the 26S proteasome in which ubiquitin, a 76-amino-acid protein, is critical for composition of monoubiquitylation and/or polyubiquitination structure through covalent attachment to a target protein. This product is organized by multistep and reversible enzymatic cascade reactions including E1, E2, and E3 enzymes [13]. The eight amino group of N-terminus or

Lysine residues (K6, K11, K27, K29, K33, K48, K63 and M1) in ubiquitin protein provide different probabilities for producing the “ubiquitination” signals [14] with diverse functional consequences (Figure 2).

Protein turnover is essential for the synaptic plasticity and memory in the nervous system, which should be considered in the regulation of protein stability and functions in neuronal cells [15]. To this end, the UPS system controls most of the protein functions in the postsynaptic response in neuronal cells. Meanwhile, it is demonstrated that protein aggregate is largely the outcome of decreasing degradation process, but not from increasing the synthesis [16]. This indicates that neurons are failed in clearing of abnormal proteins in the process of neurodegenerative proteinopathies. Thus, understanding the process of UPS system in each neuron is needed for developing novel therapeutic approaches by enhancing proteasomal degradation for removing pathogenic aggregates in neuronal cells.

2.2. Autophagy

Autophagy is another important system and conserved degradation process for supporting protein homeostasis in eukaryotic cells. Autophagy mainly processes larger cytosolic structure such as cellular protein aggregates and organelles within the lysosome [17]. The best well-known form of autophagy is macro-autophagy, in which the protein targets are sequestered in the phagophore as a cytosolic membrane compartment [18]. Mechanistically, cellular targets are swallowed by autophagosomes, which is then transported and fused with lysosomes through the cytoskeleton. This mechanism expedites the degradation of cytoplasmic substrates such as misfolding proteins (aggrephagy), high loaded peroxisomes (perophagy), abnormal mitochondria (mitophagy) and pathogenic organisms (xenophagy) [19]. In fact, several intracellular proteins are known to govern the autophagy processes, which are known as autophagy-related proteins (ATG) [20] (Figure 3).

In regard to the protective function of autophagy in the physiology of neurons, the accumulation of abnormal tau proteins might be due to impaired autophagy within neurons [21]. It was shown that elevated activation of the autophagy increases degradation of the tau protein along with reduction in intracellular tau aggregation [22]. It was also discovered that increased activity of autophagy effectively reduces A β content, particularly in the early stage of A β accumulation [23, 24]. This is in the line with the report showing that A β can be resulted from amyloid precursor protein within the autophagosome, which indicates a unique link between the autophagy pathway and the formation of A β plaques [25].

3. Chemical-mediated targeted protein degradation

The strategies of targeted protein degradation (TPD) have recently emerged as modalities in drug discovery where the bifunctional small molecules hijack the cellular degradation machinery and direct protein targets for ubiquitin-mediated destruction. In 2001, the Crews group developed the first, peptide-based, bifunctional molecules including the ligands of target protein and a ubiquitin E3, for construction of proteolysis-targeting chimera (PROTAC). Thereafter this technology has become promising for the regulation of key target proteins that are otherwise untarargetable. There are several methods have been established based on chemical-mediated targeted protein for degradation, which include

Hydrophobic Tagging; HyT, Molecular Glues, Autophagy-Targeting Chimeras; ATTAC, Autophagosome-Tethering compound; ATTEC, Lysosome-Targeting Chimeras; LYTAC, and PROTACs (Figure 3).

In HyT experimental system, the addition of hydrophobic tags (*e.g.* Boc₃Arg or Adamantyl) to ligands induce structural changes along with hydrophobic patches that initiates unfolding of protein targets for subsequent degradation via the protein homeostasis machinery systems [26]. On the other hand, in the molecular glue strategy, degraders such as lenalidomide interact the target protein with a specific, hijacked ubiquitin ligase complex for initiation of the ubiquitination process for the target protein to trigger its subsequent degradation by the 26S proteasome [27].

Moreover, Takahashi *et al.* have recently developed a novel targeted-clearance strategy termed as autophagy-targeting chimeras AUTACs, which are bifunctional molecules conjugated by small molecule that induce autophagy [28]. In brief, they added S-guanylation (guanine derivatives) as a tag to the chimeric molecules (including the guanine component and a specific ligand to a “target of interest” protein) to selectively direct protein substrates into the autophagy system for programmed destruction. They showed that AUTAC activated the removal of fragmented mitochondria and also the biogenesis of normal mitochondria. Thus, AUTAC could possibly provide a modality for developing autophagy-based drugs to its target specificity, and as an arsenal to combat Alzheimer’s disease. In addition, other strategies have been reported using autophagy including autophagosome-tethering compound, ATTEC, and also lysosome-targeting chimeras, LYTAC, for targeted protein degradation [29]. In brief, molecules of ATTEC bind to the target protein along with LC3, an autophagosome protein, that requires target proteins to the autophagosome for subsequent induction of autophagy. Meanwhile, LYTAC which is a heterobifunctional molecule, targets extracellular and also membrane-associated proteins by the conjugation that engage the endosome and lysosome by recruitment of protein targets to the lysosome-shuttling receptor in cell-surface [29].

4. PROTACs

The proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules that degrade a target protein by hijacking the powerful cellular degradation systems [30–32]. PROTACs recruit target protein to the E3 ubiquitin ligase (usually MDM2, VHL, IAPs, and CRBN ligases) with an optimal linker (Figure 4). This formation of complex between the protein of interest (POI) and the E3 ligases triggers POI ubiquitination for subsequent degradation by the 26S proteasome in eukaryotic cells [33–38] (Figure 5). The first PROTAC degrader was developed to target the androgen receptor in metastatic castration-resistant prostate cancer for treatment [39, 40]. After validation of the proof of concept, there is an intensive increase in the usage and development of the PROTAC technology in industry and academy. This is mainly because of the following reasons associated with PROTACs: 1) capable of reducing both the enzymatic and non-enzymatic functions of proteins, 2) able to destroy the “undruggable” protein of interest, 3) potent in improving specificity and selectivity, 4) elimination of protein targets rapidly and reversibly, and 5) with promise in overcoming drug resistance [41]. Drug resistance is a serious problem in

anticancer therapy, thereby PROTACs technology is going to generate more effective drugs to overcome drug resistance. The usage of PROTACs should be also a great beneficial because of its novel mechanism of action for reducing the level of the target protein using less amount of drug compared to conventional ones. The first oral PROTAC that has been assessed in clinical trials is ARV-110 for prostate cancer and also ARV-471 for breast cancer both from Arvinas [42]. In brief, PROTAC molecules can be used to cure autoimmune and inflammatory and treat diseases such allergies, asthma, cardiovascular and Alzheimer [43].

5. Proteins targeting PROTACs in the management of Alzheimer's disease

Here we introduce several PROTACs that have been developed for elimination of misfolded proteins, which is a major cause of neurodegenerative diseases. This provides an incredible therapeutic benefit to target a pathogenic protein for degradation. We will introduce the major dysregulated proteins including tau and α -synuclein that cause neurodegenerative diseases including AD. We will also discuss the main aberrant protein/signaling pathways including Hypoxia/HIF1 α signaling, PI3K/mTOR/AKT, GSK-3 β and BET that are characterized to contribute to Alzheimer disease.

5.1. The main pathological targets involved in Alzheimer's disease

5.1.1. Tau—The tau proteins are abundant in neuronal cells, which functions to stabilize microtubules in axons, thereby is known as microtubule-associated proteins (MAPs) for axonal transport [44–48]. The increased aggregation of the tau protein is correlated with synaptic dysfunction, leading to abnormal localization of tau from axons to the somatodendritic region. Neurofibrillary tangles is the aggregation of tau protein inside neuronal cells of Alzheimer's disease patients [44]. Herein, hyperphosphorylation of tau proteins develop aggregation into neurofibrillary tangles. Tauopathies which is specified by development of neurofibrils from hyperphosphorylation of tau, can arise in atypical Parkinson syndromes [49]. Dysregulation of tau is also an important issue in frontotemporal dementia (FTD), and in the toxicity of amyloid- β (A β). Thereby, tau is suggested as an attractive target for potential treatment of Alzheimer's disease.

In 2016 and also 2018, Chu and Lu *et al.* reported that using peptide form of PROTACs could degrade tau proteins [50, 51]. Interestingly, peptidic PROTAC named as TH006 could degrade tau in CA3 region of hippocampus *in vivo* (Table 1). Later in 2019, Silva *et al.* constructed a set of unique PROTACs using a tau PET tracer as a warhead [52] for targeting tau in human differentiated frontotemporal dementia (FTD) neurons. They could sufficiently degrade both wildtype (WT) and tau variants, A152T and P301L, in neurons using PROTAC T807 with the K_d value of 1.8, 2.1 and 1.7 μ M, respectively. They also established second PROTAC QC-01-175 that could specially degrade tau variants in neurons with FTD compared to healthy ones (Figure 6) (Table 1). In addition, they reported that QC-01-175 could rescue stress vulnerability in FTD patient-derived neuronal cell models.

In 2021, Wang *et al.* developed a PROTAC using a linker that could connect tau to a VHL ligand [53] (Figure 6). Through *in vitro* and *in vivo* experiments, they found that their PROTAC, C004019, could clear tau protein in both physiological and pathological conditions. Interestingly, single dose or infrequent administration of the PROTAC (once per

6 days) could sustain tau reduction and alleviate A β -induced neurotoxicity in the brain of 3xTg-AD mouse model without showing obvious abnormalities. They could also determine the robust tau clearance in hippocampus and cortex of mice along with improvement of synaptic and cognitive functions. This indicates that their PROTAC was efficient drug candidate for tauopathies and treatment of AD (Table 1).

5.1.2. α -Synuclein—The amino acid sequence of α -synuclein includes three distinct domains: N-terminus, C-terminus and a central hydrophobic region which assembles α -synuclein into amyloid fibrils [54]. The misfolded and aggregation of α -synuclein are expanded in a prion-like fashion from cell to cell, resulting the amplification and progression of the fibrils into synucleopathies [55]. Since deposition of α -synuclein initiates in the enteric nervous system, the pathogenesis of synucleopathies is assumed to begin in enteric nerves prior to deposition in the brain. In fact, patients with Parkinson's disease (PD) have gastrointestinal problems before displaying motor deficits [56]. The symptoms of PD and AD diseases are moderately similar, but pathologically AD affects the cerebral cortex and hippocampus, while PD mainly occurs in the substantia nigra [57]. Even though patients with AD show PD symptom and *vice versa*, PD patients compared to AD reveal more obvious cognitive dysfunction, suggesting that the event of pathological synergy between A β and α Syn. More studies revealed that α Syn is highly expressed in the brain region with abundant AD lesions, and the enrichment of α Syn in cortical region is correlated with existence of A β [58].

Recently, Kargbo *et al* developed bifunctional (PROTAC) compound that targets α Syn protein [59]. Their compound was developed using a Von Hippel Lindau (VHL) ends to moiety which could target protein of interest. The linker could place VHL E3 ubiquitin ligase in proximity to target protein for degradation in the UPS system. Subsequently, the representative PROTAC could 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 AD microenvironment

5.2.1. Hypoxia/HIF1 α signaling—Insufficient amount of oxygen known as hypoxia effects on pathological and physiological function of cells [60]. The prolyl hydroxylases (PHD) is recognized as a main molecular sensor of oxygen, which is disabled upon hypoxia for prolyl hydroxylation of hypoxia-induced factor alpha (HIF1 α) and also AKT [61, 62]. The hydroxylation of HIF1 α is recognized and polyubiquitinated by an E3 ligase, Von Hippel Lindau (VHL), leading to HIF1 α for further degradation by the UPS system [63, 64]. Hypoxia-driven and/or HIF1 α accumulation in *VHL*-deficient cells (ccRCCs), recruits HIF1 β for transcriptional regulation of numerous genes [64]. It was also shown that phosphorylation of histone H2AX plays an important role upon hypoxia for the maintenance and inhibition of HIF1 α degradation [65, 66].

The reduction of oxygen in the brain also associates with neurodegenerative disease such as Alzheimer's. In fact, central nervous system is highly sensitive to oxygen supply which should be due to aging that weakens delivery of oxygen *via* cardiovascular system [67]. It was also shown that hypoxia supports the formation of plaque, leading to memory deficit

in the mouse model for AD [68]. In the brain of hypoxia-driven AD, several molecular signaling is also activated to support oxidative stress [69], AKT/mTOR activation [70], angiogenesis [71], and metabolic activation. [72]. In the short period of time, hypoxia induces the expression of A β , cyclooxygenase-2 (COX-2) and presenilin 1 (PS1) for leading neuroinflammation in the brain with AD [73], but in the extended hypoxic condition, Ca²⁺ channels are upregulated for production of A β [74]. Importantly, HIF1 α triggers transcriptional expression of VEGF [75] and BACE1 (main biomarker for AD) [76], thereby increasing A β deposition and also neurotic plaque production in transgenic mouse model [76]. This indicates that HIF1 α induces tau phosphorylation in part through regulation of several signaling pathway including GSK3 β , CDK5 and mTOR [77, 78]. Moreover, Glut1 and Glut3, other HIF1 α -target genes and the main brain glucose transporters, are expressed less in AD [79].

In early 2004, Schneekloth JS Jr *et al.* reported peptide-based PROTACs could recognize the transcription HIF1 α and had also the potentiality to bind to VHL [80]. They constructed a poly-D-arginine tag on the peptide sequence which could assist in cell penetration. Interestingly, the discovery of small-molecule that could inhibit the interaction between HIF1 α and VHL, initiated the establishment of several PROTACs, which link VHL to other target protein of interest with high specificity and high-affinity [81, 82]. Taken together, aging could induce hypoxia/HIF1 α for dysregulation of several molecular signaling in neurons for AD activation, which should be considered for careful and selective degradation using novel PROTACs technology.

5.2.2. PI3K/mTOR signaling—Phosphatidylinositol 3-kinases (PI3K) is a member of the PI3K/AKT/mTOR signaling pathway functions as phosphatidylinositol kinases. It mainly regulates apoptosis, proliferation and differentiation of cell and its overexpression drives the main feature of tumorigenesis. Moreover, the mammalian target of rapamycin (mTOR) responds to wide range of extracellular stimuli for the regulation of cell growth and metabolic homeostasis [83]. mTOR pathway also regulates several diseases including cancer [84], diabetes [85] and neurodegenerative pathological condition [86]. In fact, inhibiting mTOR expands life span of several organism such as *C. elegans* [87] and mice [88]. Constantly, Rapamycin as a mTOR specific inhibitor extends their lifespan [89–93]. Importantly, mTOR kinase promotes the phosphorylation of tau by regulating multiple kinases including glycogen synthase kinase 3 (GSK3), protein kinase A (PKA), and cyclin-dependent kinase 5 (CDK5) [94]. Moreover, mTOR directly phosphorylates and inhibits protein phosphatase 2A (PP2A), which is a main phosphatase downregulated in the brain with AD [95], for increasing tau phosphorylation [96]. Furthermore, downstream targets of mTOR such as S6K and eukaryotic translation factor 4E (eIF4E), elevate mRNA translation level of Tau, indicates that overactivation of mTOR could accumulate tau protein [97]. Surprisingly, the administration of A β in the hippocampus of normal mice could activate the mTOR pathway, representing the role of the amyloid precursor in the activity of mTOR signaling [98]. Furthermore, rapamycin could reduce cognitive deficits in the tau pathology through amelioration of A β in the AD brains of mice [99]. Taken together, mTOR imbalances the homeostasis of tau, resulting in the aggregation of tau and formation of

neurofibrillary tangles for AD generation. This suggests potential strategy for treatment of AD through targeting mTOR.

Recently several PI3K inhibitors have been established in which most of them have limitation because of less selectivity and also side effects [100, 101]. It means that improvement of unique PI3K-targeting PROTACs has been recognized as potent strategy. In 2018, Jiang *et al.* has established a set of prospective PI3K-degraders using lenalidomide links to the ZSTK474 as a PI3K inhibitor [102]. Although this PROTAC revealed less enzymatic activity compared to ZSTK474, they could successfully degrade PI3K at 10 μ M along with decreasing the phosphorylation of GSK-3 β , S6K and AKT in the PI3K/AKT/mTOR signaling pathway.

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

In 2019, Toker *et al.* developed a specific small molecule degrader using the conjugation of CRBN ligand along with GDC-0068 as an AKT inhibitor [105]. This engineered PROTAC could accordingly inhibit AKT1, AKT2, and AKT3 with IC₅₀ values at 2.0 nM, 6.8 nM, and 3.5 nM, whereas IC₅₀ values of GDC-0068 was measured at 5 nM, 18 nM, and 8 nM, respectively. In addition, this PROTAC could destabilize all three isoforms followed by reduction in AKT-downstream signaling. They also found that the engineered PROTAC could downregulate cell proliferation much efficient than its parental inhibitor, suggesting its beneficial for targeted degradation of AKT.

5.2.4. GSK-3 β —Glycogen synthase kinase 3; GSK-3, is known as serine/threonine protein kinase and a member of phosphotransferase family [106]. Pathologically, GSK-3 β regulates several process through phosphorylation of tau and also produce amyloid beta peptide which cause neurofibrillary tangles and amyloid in Alzheimer's disease [104]. Moreover, proinflammatory function of GSK-3 β causes the loss of neuron [107–109] and develop neurodegenerative disease [110], thereby should be considered as therapeutic target to fight against AD [111–113]. In 2021, Jiang *et al.* reported a first set of GSK-3 β targeting PROTACs which was developed using CRBN as an E3 ubiquitin ligase [114]. Their PROTAC could efficiently induce degradation of GSK-3 β protein (44%) in less IC₅₀ value at 2.8 μ M, which should be considered as effective GSK-3 β degraders in the PROTAC system.

5.2.5. BET—Dysregulation of inflammation is critical process in the pathology of many CNS diseases, which occurs through several pathways including NF- κ B and Nrf2 signaling involved in inflammation. Extra-terminal domain (BET) proteins include four members (BRD2, BRD3, BRD4 and BRDT) that play critical roles for transcriptional regulation of

inflammatory response [115, 116]. For example, BETs assemble the complex of histone acetylation-dependent chromatin for the expression of inflammatory genes. Early studies have also shown that inhibition of BET activates anti-inflammation, suggesting that BRD2, BRD3 and BRD4 proteins could play critical roles in AD and other neuroinflammation disorders. [116–120]. Targeting BET proteins using small-molecule inhibitor (JQ1) downregulates several pro-inflammatory regulators such as IL-1 β and TNF- α followed by the phosphorylation of tau at Ser396 in the frontal cortex and the hippocampus of the 3xTg mouse model for AD [121]. However, mice did not show deficits in memory and the ameliorating in learning. In the AD mouse model of the APP/PS1, JQ1 could enhance long-term potentiation and cognitive function [122]. Further study showed that JQ1 could activate the gene expression of hippocampal genes, which were responsible to the activation of ion channel and DNA repair [122]. The chemical probes such as pan-BETi(s) serve as an ideal PROTAC for targeting BET proteins due to their potentiality for identification and recruitment of BET.

The first PROTACs targeted BET proteins (dBET1) were identified in 2015 and contained by an E3 ligase and a BRD4 BD binding moiety (JQ1 or OTX015) [123]. The BET PROTACs could identify and recruit the CRBN E3 ubiquitin ligase for efficient and selective degradation of BRD4 protein *in vitro* and *in vivo* [123–127]. Moreover, dBET1 could eliminate BET proteins 8–10-fold along with several other BET-target proteins revealed by the proteomic study [123].

6. The Strengths and weaknesses of PROTACs Technology

6.1. Advantages

The PROTACs technology has several advantages over traditional approaches that make them exclusively suitable for the treatment of CNS diseases [128]. The main advantage is the capability of PROTACs for utilizing targeted degradation of undruggable proteins in the CNS. The specificity of PROTACs is high in comparison to other methods, in which they can selectively degrade different isotopes of proteins which are expressed by the same gene. Furthermore, PROTACs bypass the potential toxic effects that can be developed by pharmacological approaches. In fact, they do not need to directly attach target protein for long period of time for inactivation of the target protein function. Moreover, development of PROTACs is designed based on catalytic reaction, which can be reused for many cycles until the target proteins in cells are eliminated. Furthermore, PROTACs have sub-stoichiometric catalytic activity [129], letting the administration of very low concentrations to be sufficient enough to degrade a target protein. Therefore, there is strong feasibility to develop an active drug using PROTACs, in which the inhibition of the target protein is not required molar mass of drug [130, 131]. Overall, these notions suggest that PROTACs could be a very effective method to target and destroy undruggable proteins that is depictive in the pathophysiology of many CNS disorders including AD.

6.2. Disadvantages

There are a number of limitations that could prevent development of PROTAC drug for the clinical applications in the future. Because PROTACs technology employ drugs for

dual targets, thereby 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 issue, which reveal a serious barrier in pharmacokinetics. For example, one main issue is the blood-brain barrier permeability, which is a limiting factor for many pharmacological approaches. As we reviewed the efficacy study of PROTAC named as C004019 (section 5.1.1) [53], it is noteworthy to suggest that they might also effectively penetrate the blood-brain barrier [30].

Another possible limitation is localization of the PROTAC to the specific brain regions, which are aimed to combat neurodegenerative diseases such as Alzheimer's disease. For successful use of PROTAC, the E3 ligase must be expressed in the target region(s). However, some E3 ligases (*e.g.* CRBN) [132] are differentially expressed across brain areas. This differential expression pattern can complicate treating diseases. For example, tau accumulation occurs in a progressive manner in the AD brain regions that is started in locus coeruleus and also entorhinal cortex and ended in primary visual cortex [133]. This suggests that depending on disease progression, PROTACs would need to target tau precisely in some specific regions of the brain. Therefore, it is challenging to manage drug for colocalization with and/or without expression of E3 ligase in the affected brain regions, and it needs establishment of new technology to solve this emerging concern [134]. Furthermore, it was shown that many CNS disorders are associated with expansive reductions in proteasome catalytic activity [135]. This indicates that even if a PROTAC can ubiquitinate the protein of interest, it still may not possibly degrade it. Thus, reduced proteasome function remains a barrier for PROTAC-mediated degradation of target proteins for treatment of AD, which are divergently expressed in the brain.

7. Discussion

PROTACs is a powerful and attractive strategy studied and developed in both academy and industry. Recently, they have been widely investigated for treatment of several diseases including cancer, neurodegeneration, immune disorders, cardiovascular dysfunction, fatty liver disease (FLD), and for controlling viral infections. Here we discuss the AD-specific PROTACs, and also several others that have been used for targeting aberrant proteins/signaling which are dysregulated in tumor microenvironment. However, due to their similar structure and mechanism, we introduce them as potential PROTACs to fight against AD. We reviewed two peptidic PROTACs that have been developed for targeting tau in treatment of AD [50, 51]. Although they could degrade tau in the center of the aberrant signaling in AD, their application has been limited in part due to their intrinsic weakness against protease degradation and their poor membrane permeability *in vivo*. The stability of those peptides might be improved by substitution of unnatural amino acid, backbone modification, and cyclization [136] and their cell membrane permeability could be modified by increasing lipophilicity and decreasing hydrogen bonding [137]. Silva *et al.* have also established another type of PROTACs using a tau PET tracer as a warhead, however, their application was not validated *in vivo* [52]). In fact, the construction of PROTAC such as warhead with high affinity to target protein might be not enough for the generation of an active PROTAC [138]. Therefore, the induction of steric structure by the formation of target protein linked to hijacked E3 ligase could facilitate the transfer of ubiquitin from E2 to target

protein and potentiate PROTAC activity. This ternary complex structure enables PROTAC to degrade large molecules such as protein aggregates, which are normally not allowed in the proteasome's barrel-like structure for degradation [15]. Through this approach, Wang *et al.* established PROTAC, named as C004019, that could efficiently induce clearance of tau in the brain of hTau and 3xTg-AD transgenic mice. This indicates that C004019 could potentially pass through the proteasome's barrel-like structure and also the brain-blood barrier, in which most of large drugs unable to do so. Although C004019 could remarkably improve synaptic and cognitive functions in the mice, its selectivity was revealed poor for recognition of tau species (wild type and phosphorylated tau). This might be in part due to the structure of tau which is natively unfolded protein, thereby opening new challenge for the improvement of their PROTAC capability.

Although aging is a main cause of cancer and AD [139], there are some studies revealing pathogenesis of AD protects patients against cancer and *vice versa*, but the underlying mechanisms remain elusive to date [140, 141]. Notably, cancer and AD pathologically share common feature, for example, a high level of cell cycle re-entry, which is required for cancer pathogenesis, is also revealed in the AD patients [142, 143], but instead of cell division, neuronal cell cycle is aborted going to death [144, 145]. This includes a significant elevation in the activation of CDK2, CDK4, CDK5 and caspases during cell cycling for APP phosphorylation, APP proteolysis, and production of A β [146–148]. The role of mTOR activation for the initiation of proliferation suggests its function in the neuronal cell cycle re-entry and pathogenesis of AD [149]. The activation of mTOR is also believed for driving the neurodegeneration through activation of Tau [150], leading to the accumulation of NFTs. Moreover, inactivation of mTOR pathway results in the activation of autophagy for cell clearance from A β [151]. In the brain tumor microenvironment, the adenosine monophosphate protein kinase protein (AMPK) might be activated upon energy stress for positively activation of FOXO3 [152] to protect neuronal cells. This energy stress is due to high metabolomic rate of cancer cell proliferation that hijacking energy in the brain microenvironment. Meanwhile, the mTOR signaling and the consequent activation of cell cycle re-entry is inhibited by AMPK [153], in which FOXO increases the expression of antioxidant enzymes to diminish the damage of cell [154, 155] and inhibit neurodegeneration. Taken together, mTOR-mediated cell growth and proliferation not only drives cancer cell progression, but also causes neuronal cell arrest that is a readout of disabled cell division in neuronal cells. Therefore, targeting the PI3K/mTOR/AKT axis introduces a great therapeutic option for the management of AD and tumor microenvironments (Figure 7).

In section 5.2, we briefly introduced several PROTACs that have been established to target HIF1 α , PI3K, AKT, and BET in tumor microenvironment with aberrant signaling. Most of these PROTACs could efficiently degrade their own target proteins, such as BET, with high sensitivity and selectivity. Therefore, the application of these PROTACs shows great potential to target AD microenvironment. Although cancer and AD are pathologically share common feature, the disease-causing mechanisms differ dramatically. Given that most established PROTACs were mainly studied in the cancer disease setting, their formulation and mechanism of actions should be carefully designed to combat AD. Currently, two PROTACs, ARV-110 and ARV-47, have been used in clinical trials for treatment of prostate

and breast cancer, respectively by targeting of androgen receptor and estrogen receptor (from Arvinas). They both show an acceptable safety trial in which ARV-471 is well-tolerated at all tested dose levels without serious side effects. Interestingly, ARV-471 could produce a synergistic effect on the inhibition of tumor in the combination treatment using kinase inhibitors such as CDK4/6 inhibitors. This indicates that combination of PROTAC with chemotherapy, antibody (in immunotherapy), and small molecule inhibitors might be an alternative approach for treatment of disease, including cancer and AD.

8. Conclusion and Perspectives

The PROTACs technology enables us to discover new therapeutic agents with unique capability for degradation of “undruggable” proteins instead of inhibition. They are capable of answering some concerns related to the use of traditional small molecules which have poor selectivity, side effects along with drug resistance. However, the toxicity of PROTACs can limit future drug development in part due to the effects of off-target degradation. The PROTACs degrade proteins completely in the ubiquitin proteasome system, while parental small molecules or compounds are just required to inhibit protein functions. Therefore, the combination of small molecules with a tissue specific E3 ligase provided great advantages for selective degradation of target proteins. Moreover, the structure of PROTACs is generally complex and their syntheses are complicated because of high molecular weight (800–1000 kDa), which should be recognized for further modification and screening for the improvement of the brain membrane permeability. This needs further optimization in the structure and synthesis of PROTACs, which is essential in the stable platform for drug discovery. To this end, the application of crystallography can help us to understand their structural mechanism. For example, structural analyses of VHL and/or Cereblon E3 ligase proteins demonstrate the shape and the position of the Cullin-RING E3 ubiquitin ligase along with E2 and target proteins in details that enable us to target ubiquitin transfer [156].

As we mentioned, it is impossible to confirm that all proteins of interest will be degraded by the PROTAC in the absence of the ligands. Therefore, selection of new ligands and validation of targets will be another focus in the PROTAC development. There are approximately over six hundreds of E3 ligases existing in the human genome, but only a few of them have been used in PROTACs technology. This shows the possibility for expanding effort to screen and find suitable E3 ligases for this issue. Furthermore, more E3 ligases need to be investigated for avoiding off-target in timely response to brain diseases especially in Alzheimer’s disease. Finally, recent advancements in the CRISPR-dCas9 technology could be helpful for stimulation of proteasome function in the brain disorders with expansive reductions in proteasome catalytic activity [157]. Interestingly, it was found that IU1, as a compound for inhibition of ubiquitin-specific protease 14 (USP14), could improve protein degradation *in vitro* [158] and in mouse brain tissue [159], although proteasome function was not broadly enhanced in the brain [160]. This suggests a viable avenue in careful designing of combination therapy using PROTACs, monoclonal antibodies, RNA interferes, and/or small-molecule inhibitors to combat Alzheimer’s disease.

9. Data Retrieval

This review was accomplished using electronic databases, such as PubMed, Scopus, Web of Medline for finding articles linked to Alzheimer's disease, 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 entitled principles were PROTACs affecting aberrant signaling pathway in Alzheimer's disease. Data were gathered without date limitations until November 2021. The list of reference in the articles were also reviewed by the hand search for aberrant signaling pathways, which was recognized as a crucial therapeutic target in AD.

Acknowledgments

We would like to sincerely thank our colleagues for critical reading of the manuscript. We are also very sorry that due to space limitation, we could not include all of the studies related to Alzheimer's disease and PROTACs in this review. This study was partially supported by US National Institutes of Health (NIH) grants to W.W. (R35CA253027).

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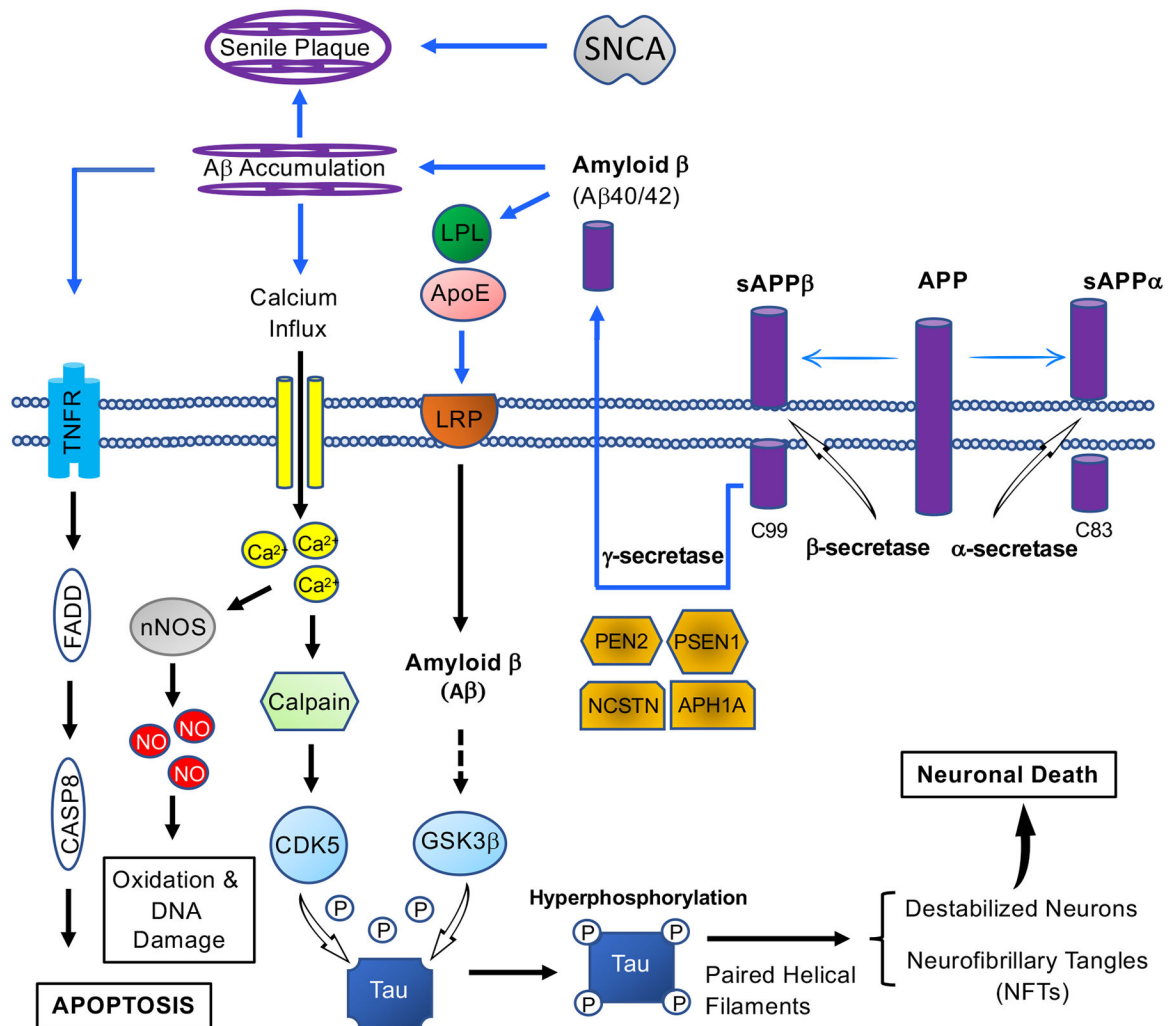


Figure 1. Therapeutic targets in Alzheimer's disease signaling.

The formation of NFTs and senile plaques in AD signaling introduces several therapeutic targets. Four genes are pathogenetically committed to AD: amyloid precursor protein (APP), apolipoprotein E (ApoE), presenilin 1 (PSEN1) and presenilin2 (PSEN2). An increased level of amyloid-beta peptides is caused by mutations in APP and PSEN, leading to the formation of amyloid-beta 42, the main component of senile plaques. Cleavage of APP either by 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 amyloid-beta proteins, Amyloid β ($A\beta_{40/42}$). In neuronal cell bodies, neurite outgrowth is stimulated by ApoE-containing lipoprotein lipase (LPL), via interacting with and letting LRP to bind APP for production of proteolytic fragment ($A\beta$) [161]. The accumulation Amyloid β results in blocked ion channels, mitochondrial oxidative stress, and activation of TNFR-regulated Caspase 8, and ultimately neuronal cell death. On the other hand, GSK-3 phosphorylates Tau at several sites for partial inhibition of tau biological activity in AD [162]. Upon abnormal Ca^{2+} homeostasis disturbance, the stimulation of calpain mediates the cleavage of p35 to p25 for the activation of CDK5,

leading to hyperphosphorylation of Tau, and also promotion of APP truncation [163]. Finally, the elevated Ca^{2+} stimulates neuronal NO synthase, leading to production of nitrogen species and reactive oxygen [164]. SNCA: Alpha 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 oxides synthase

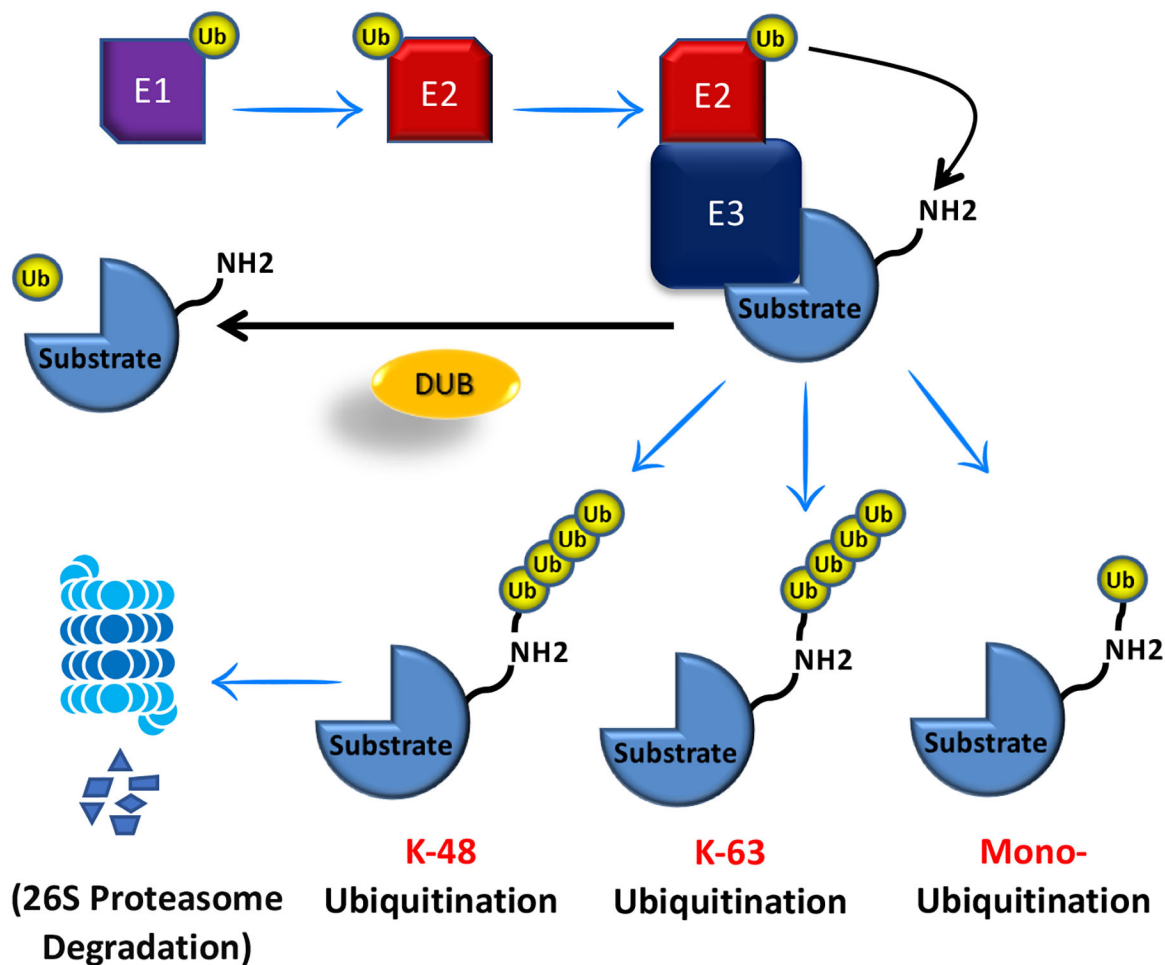


Figure 2. A schematic representation of the E3 ligase biology.

The ubiquitination of cellular proteins is triggered by the E1 enzyme that utilizes the formation of Ub-AMP. Following this catalytic reaction, ubiquitin is subsequently transferred to an E2 enzyme for activation of the thioester-linked E2-Ub complex. Finally, an E3 ligase enzyme transfers the ubiquitin protein from the E2 enzyme to a target protein. This ubiquitination process can be reversed by deubiquitinating enzymes (DUBs), which catalyze the cleavage of ubiquitin from target protein or substrate.

E1, Ubiquitin-activating enzymes; E2, ubiquitin-conjugating enzymes; and E3, ubiquitin ligases and Ub, Ubiquitin.

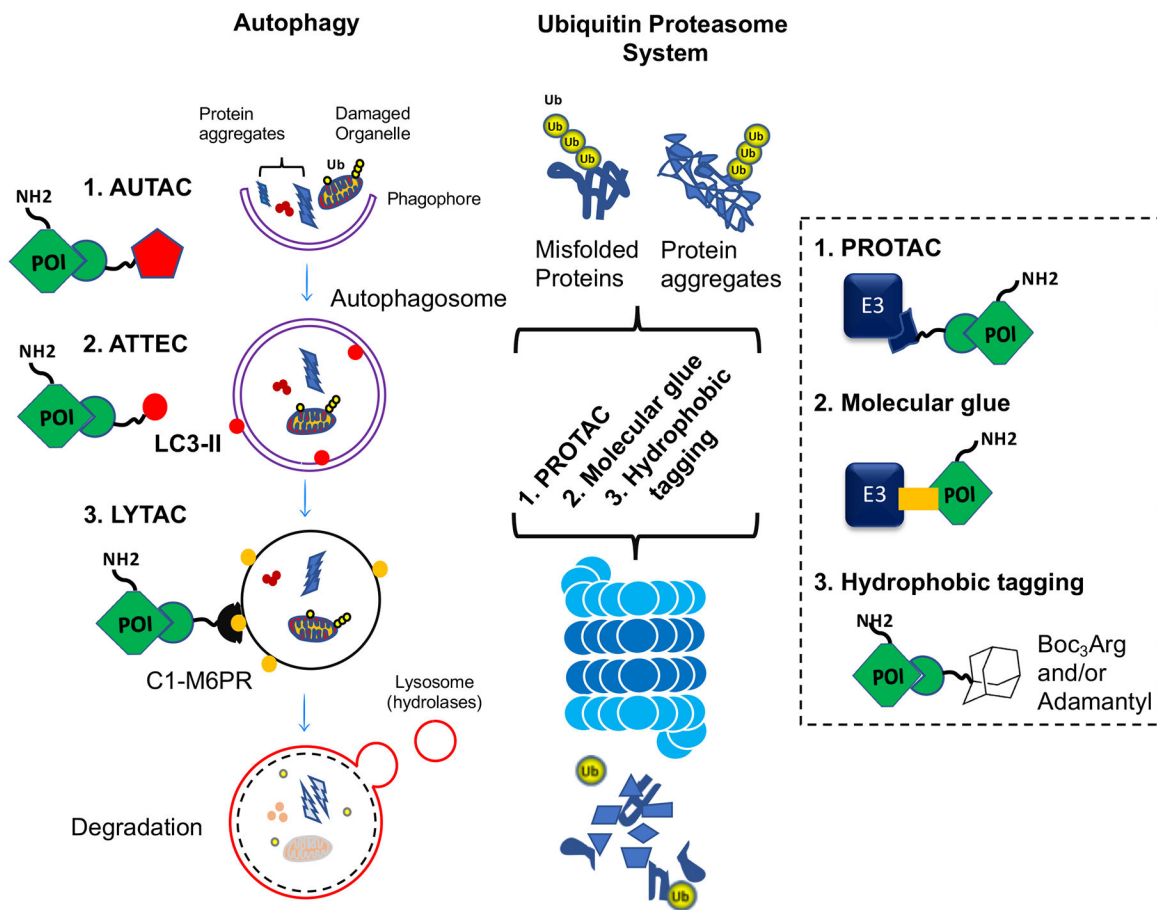


Figure 3. Protein degradation pathways in the neuronal cells along with targeted protein degradation strategies. PROTAC, proteolysis-targeting chimera; AUTAC, autophagy-targeting chimeras; ATTEC, autophagosome-tethering compound; LYTAC, lysosome-targeting chimeras; C1M6PR, cation-independent mannose 6-phosphate receptor; LC3, Microtubule-associated proteins 1A/1B light chain 3B Ub, ubiquitin; E3, ubiquitin ligase; POI, protein of interest.

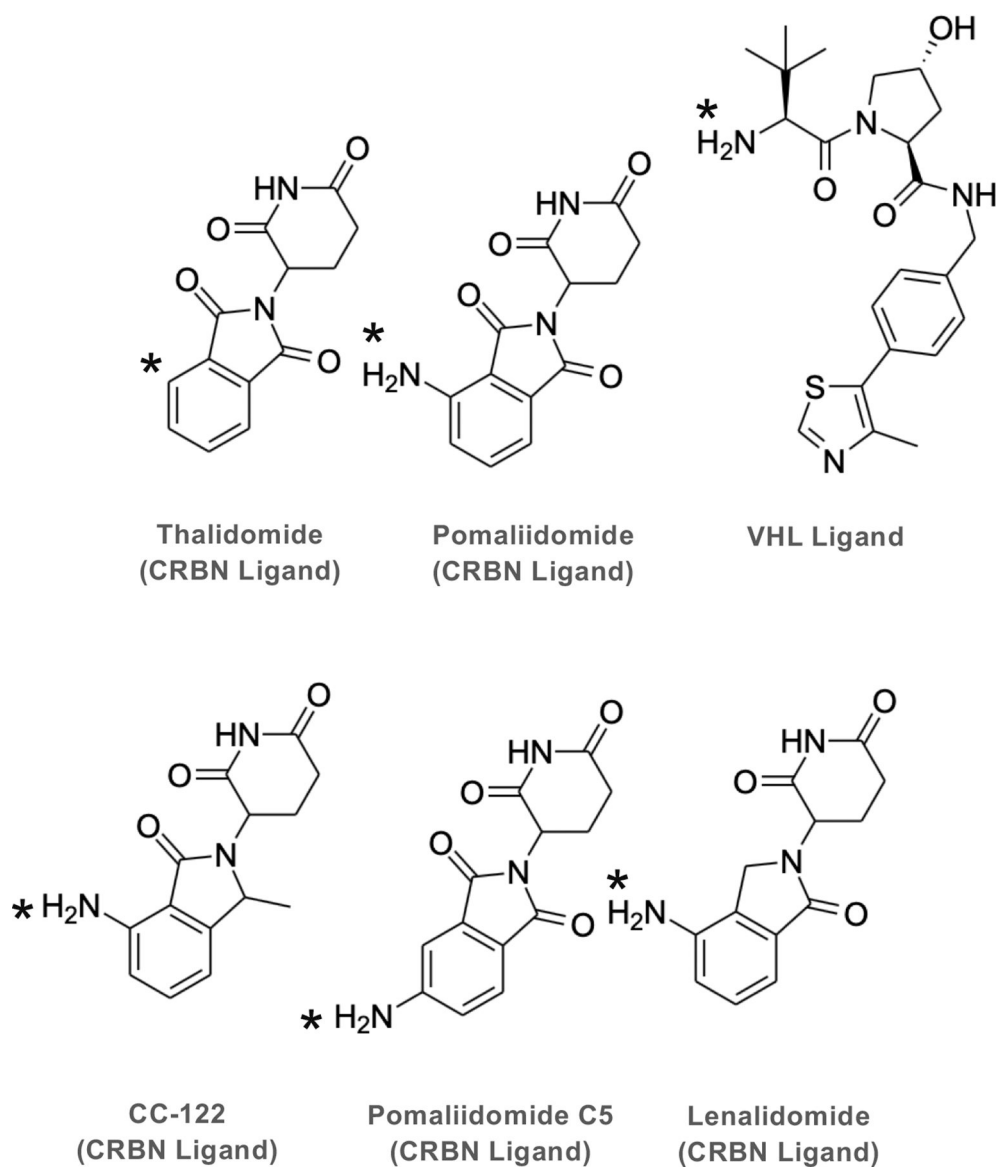
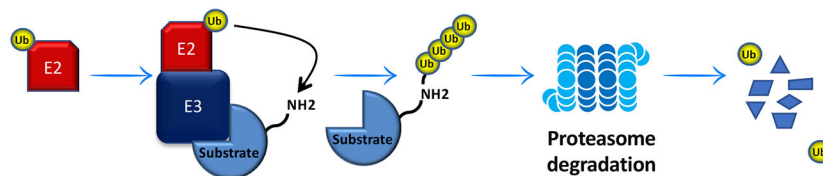


Figure 4. E3 ligase ligands that are commonly used for PROTACs. Derivatives of Thalidomide targeting Cereblon (CRBN) and the ligand of Von Hippel Lindau (VHL). The asterisk shows the point of attachment to the linker.

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



B. PROTAC-induced degradation of POI through UPS

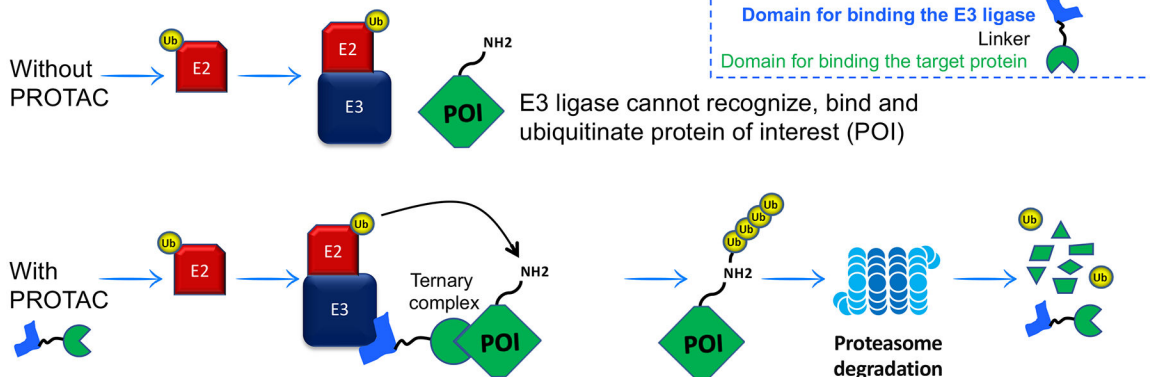


Figure 5. A schematic illustration of PROTAC mechanism(s) of actions.
 The E1 Ubiquitin-activating enzyme initiates transferring of Ubiquitin (Ub) to a target protein through the E1-E2-E3 enzymatic cascade. Then, the ubiquitinated target protein is degraded by the 26S proteasome. Without PROTAC, target protein is not recruited for the process of ubiquitination. The molecule of PROTAC attaches protein of interest (POI) with the E3 ubiquitin ligase for ubiquitination and subsequent proteasomal degradation.

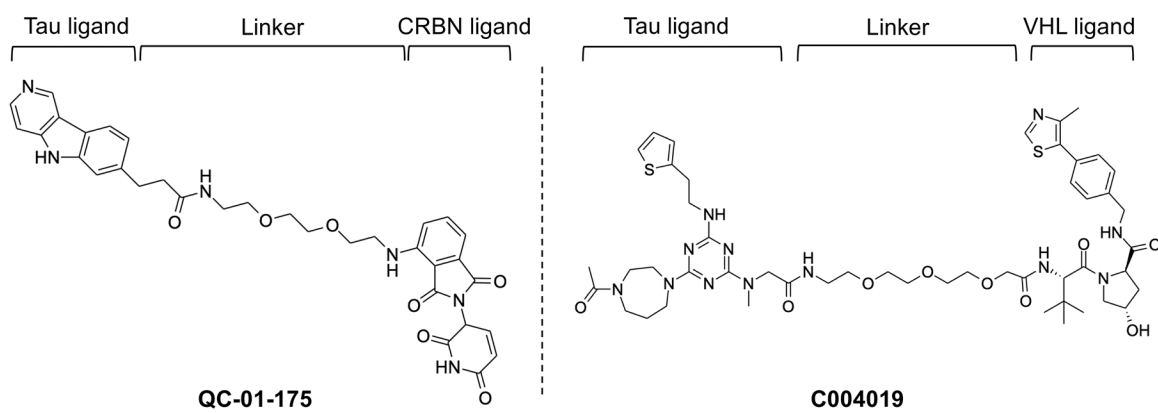


Figure 6.
Chemical structure of Tau targeting PROTACs, QC-01-175 and C004019.

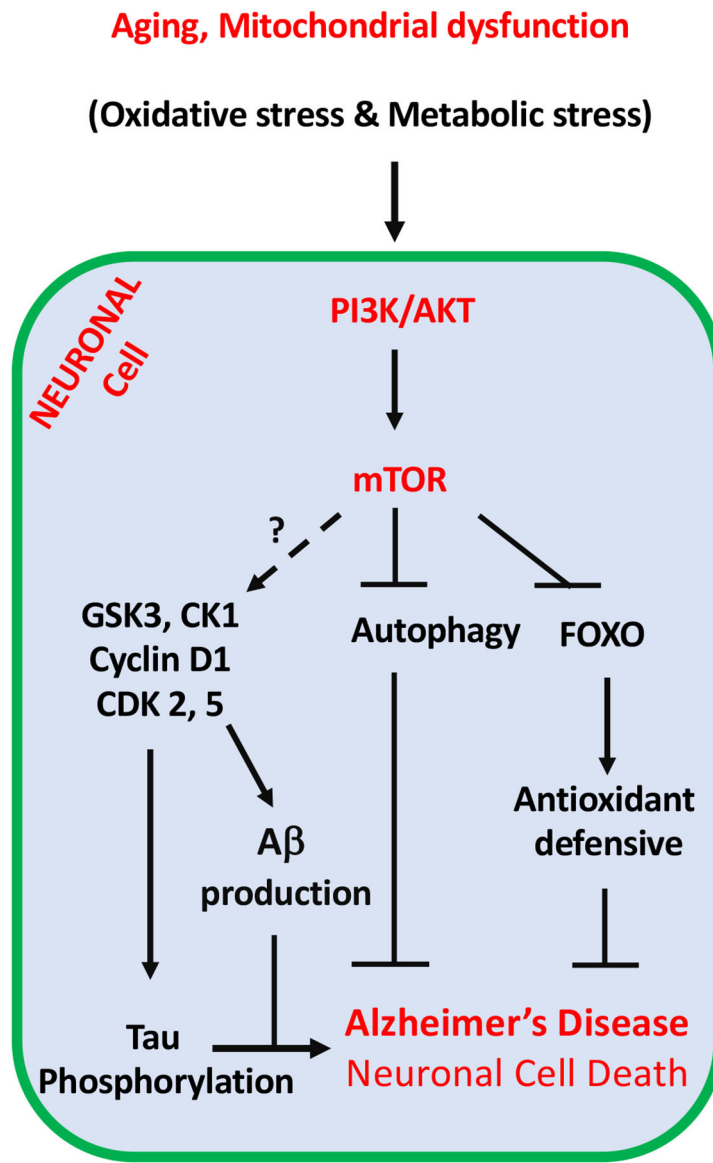


Figure 7. Therapeutic potential of the PI3K/AKT/mTOR axis for the management of AD and cancer.

A long-term function of physiological stimulators such as aging followed by mitochondrial dysfunction might accumulate oxidative stress and metabolic stress, which both trigger the activation of the PI3K/AKT/mTOR signaling for highly activation of cell cycle re-entry in neuronal cells. In contrast with cancer, neuronal cell cycle is aborted and going to death instead of cell division. Moreover, a significant elevation of CDK2, CDK4, CDK5 and activation of caspases during cell cycle re-entry leading to APP phosphorylation, APP proteolysis, and production of A β . Furthermore, the activation of mTOR pathway results in the inactivation of autophagy, which is required for the clearance of A β in the neuronal cells. Upon cancer cell proliferation in the brain and hijacking energy from neurons, AMPK is stimulated upon this energy stress and positively activate FOXO3 to protect neuronal cells in the brain tumor microenvironment. FOXO increases the expression of antioxidant enzymes

to diminish the damage of neuronal cell. Dashed arrow required additional investigation for that molecular network in the certain condition.

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Table 1.

A description of the properties for peptide- or chemical mediated targeting PROTACs.

PROTAC	Entityc	Recognize Tau	Linker	Recruit E3 Ligasea	Cell-Penetrating Peptide	IC ₅₀ (μM)	K _d value (μM)	In Vivo study	PK study	PMID
Peptid-mediated targeting	TH006	YQQYQDATADEQG	GSGS	ALAPYIP (VHL)	RRRRRRRR ^a	None	0.3944 ± 0.1589	Tau degradation in CA ₃ region of hippocampus	Not reported	27105281
	Peptide 1	YQQYQDATADEQG	GSGS	LDPETGEYL(Kcap1)	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}	1.8 ± 0.99						
		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 hippocampus and cortex; Improvement of synaptic and cognitive functions	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

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