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Autophagy receptor-inspired chimeras: A novel approach to facilitate the removal of protein aggregates and organelle by autophagy degradation

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Abstract: Neurodegenerative diseases are genetic disorders of the central nervous system. Their key feature is the slow accumulation of misfolded protein deposits in brain neurons. While autophagy is known to play a crucial role in degrading protein aggregates, there is currently no effective and widely applicable method for the degradation of protein aggregates in mammalian cells. The latest data demonstrate that synthetic autophagy receptor-inspired targeting chimeras (AceTACs) act as degraders that can combine the LIR domain of the selective autophagy receptor-p62 with antibodies. Different protein aggregates (such as mHTT, TDP-43, and Tau) can be selectively targeted for disintegration by AceTAC degraders. Moreover, these degraders can target intracellular organelles including mitochondria, peroxisomes, and endoplasmic reticulum. Therefore, AceTACs represent promising autophagy-based targeted degraders that could offer a novel approach to effectively treat neurodegenerative diseases..

Key words: Synthetic autophagy receptors; Autophagy; p62; Protein aggregates; Organelles

Neurodegenerative diseases (NDDs), mainly including Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), are sporadic and rare genetic disorders of the central nervous system. A key feature of these conditions is the slow accumulation of misfolded protein deposits in brain neurons, the excessive aggregation of which leads to neurotoxicity and further disorders of the nervous system.

Under physiological conditions, the polyglutamine (poly Q) of huntingtin protein (HTT) consists of no more than 30 glutamines. However, due to the mutation of HTT gene, too long poly Q will lead to the abnormal aggregation of mutant HTT (mHTT), forming harmful protein precipitation and leading to HD (Llamas et al., 2023). ALS is another rare progressive NDD. Pathological protein aggregates, such as TAR DNA-binding protein 43 (TDP-43), superoxide dismutase 1 (SOD1), and sarcoma fusion protein (FUS), contribute to the development of ALS. In addition, dipeptide repeat (DPR) protein aggregates produced by the expansion of the chromosome 9 open reading frame 72 (C9ORF72) repeat sequence also promote the process of ALS (Kim et al., 2020). As for AD, the abnormal aggregation of amyloid-β and neurofibrillary tangles are two critical pathogenic factors (Busche et al., 2020). Studies consistently reveal that abnormal protein aggregates are involved in the development of HD, ALS and AD. Therefore, effectively removing these toxic protein aggregates becomes an extremely important therapeutic goal for the treatment of HD, ALS and AD.

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Recent reports highlighted that autophagy plays a vital role in the degradation of these excessive protein aggregates, thereby alleviating the development of HD, ALS and AD (Zhang et al., 2021). Conversely, autophagy is usually impaired in the pathogenesis of these conditions. In fact, the inhibition of autophagy promotes the accumulation of these abnormal protein aggregates. Disrupting the interaction between p62 and microtubule-associated protein light chain 3 (LC3) has been shown to result in the accumulation of mHTT, ultimately aggravating HD (Yang et al., 2021). Moreover, p62 gene mutations, which lead to impaired autophagy, accelerate the accumulation of TDP-43, and promote the development of ALS (Barmada et al., 2014). In AD mice, autophagy is also blocked and tau protein can further build up, aggravating the severity of the disease (Bourdenx et al., 2021). On the contrary, current researches show that autophagy-dependent protein degradation techniques, such as autophagy-targeting chimera (AUTAC) and autophagosome-tethering compound (ATTEC), can enhance the clearance of abnormal aggregated proteins and alleviate the development of HD, ALS and AD (Li et al., 2019; Takahashi et al., 2019; Tan et al., 2023). Therefore, promoting targeted protein degradation may greatly improve the therapeutic effects of HD, ALS and AD.

Targeted protein degradation technologies that have made important progress in recent years can be mainly divided into two categories: small-molecule conjugated chimeras (such as AUTAC and ATTEC) and antibody-conjugated chimeras (such as antibody-based PROTAC and GlueTAC). The former are characterized by good bioavailability and cell membrane permeability. However, they have poor specificity and thus lack precise targeting capabilities for target proteins or organelles. On the other hand, antibody-conjugated chimeras can not only improve the specificity and targeting of chimeras but also prolong their biological half-life. On the downside, antibody-conjugated chimeras have rather high development and production costs, as well as relatively slow metabolism and clearance from the body, which may increase the risk of long-term toxicity (Dragovich, 2022).

P62, the major mammalian selective autophagy receptor, contains multiple domains including ubiquitin-associated domain (UBA), phox and bem1 (PB1), LC3 interacting region (LIR), and zinc finger domain (ZZ). The UBA domain binds to ubiquitination substrates to form aggregates, and the PB1 domain also participates in the formation of aggregates through self-oligomerization. After the auto-lysosomal fusion, the aggregates are targeted to the phagophore through the LIR domain for further degradation. Besides, the ZZ domain of P62 can also induce autophagy by binding to N-terminal degraders (Lin et al., 2013). (Fig. 1a). Based on the structure of p62, Ji et al. developed the AUTOTAC technology, which simultaneously binds the target protein and the ZZ domain of p62, promotes the activation of p62, and degrades the target protein (Ji et al., 2022; Lee et al., 2023). Recently, also based on the structure of p62, Jiang et al. (2023) developed a series of new targeted degraders (AceTACs) by conjugating the LIR domain of autophagy receptor with antibodies. On the one hand, the LIR domain of AceTACs can bind with LC3 on the autophagosome membrane. On the other hand, the antibodies of AceTACs can target specific substrates (such as mHTT, TDP-43 and Tau proteins). Furthermore, the above substrates can drive to form autophagosomes through interaction between the LIRs of AceTACs and LC3 on the autophagosome membrane, ultimately promoting the selective degradation of the target-specific substrates (Fig. 1b).

Jiang et al. connected the target-specific antibodies to the C-terminus of p62 (p62FL-An, n represents the number of antibodies) to form AceTAC degraders. They used mHTT protein as a degradation target to detect the degradation efficiency of p62FL-An. Their experimental results showed that p62FL-An (p62FL-A1, p62FL-A2 and p62FL-A3) successfully targeted and degraded mHTT. Different types of antibodies have different degradation efficiencies. For example, p62FL-A1, p62FL-A2, and p62FL-A3 all reach 50% degradation efficiency. To improve the degradation efficiency of AceTAC degraders and further determine the key structures affecting AceTAC degradation efficiency, they generated five domain/motif truncations of p62FL-A3. They found that the LIR motif in p62FL-A3 could be further refined to the TP53INP2 motif, and the degradation efficiency of mHTT aggregation reached the highest level (about 72%). These results suggest that TP53INP2 motif in AceTAC degraders can modulate mHTT degradation efficiency. Consequently, for AceTAC degraders, the degradation efficiency of mHTT can be improved by changing the number of

TP53INP2 motifs and the number of antibodies (ΔN-TmAn, where m represents the number of TP53INP2 motifs). Particularly, ΔN-T3A3 can achieve about 90% of the target degradation of mHTT. The above results highlight that AceTAC degraders can successfully target and degrade mHTT aggregates. Moreover, changing the number of TP53INP2 motifs and antibodies can significantly regulate the degradation efficiency.

Similar to mHTT aggregation, TDP-43 and Tau can also aggregate and become neurotoxic proteins. Jiang et al. further verified the possibility of AceTAC degrading TDP-43 and Tau aggregations by designing AceTAC degraders that target TPD-43 and Tau mutants. These AceTAC degraders could break down TDP-43 and Tau aggregations, which further expands their applicability.

Beyond toxic protein aggregates, failure to clear damaged or useless organelles, which may result in cell dysfunction or death, has also been linked to various diseases. For example, the blockage of pathways that regulate mitochondria, the power source of nerve cells, can lead to the accumulation of damaged mitochondria, causing gradual neuronal death and ultimately divergent Parkinson's disease (Magalhaes et al., 2021). Jiang et al. (2023) further explored whether AceTAC degraders could be extended to organelles, such as mitochondria, peroxisomes, and endoplasmic reticulum (ER). They used translocase of the outer membrane 20 (TOM20), peroxisome biogenesis factor 3 (PEX3), and cytochrome P450 (CYP450) as recognition sites of mitochondria, peroxisomes and ER, respectively. Next, they confirmed the feasibility of AceTAC targeting mitochondrial peroxisomes and ER degradation through a variety of biological methods such as flow cytometry and confocal cell localization. They showed that as the number of recognition sites on the organelles increases, the degradation efficiency of AceTAC degraders can be further improved. Overall, these results indicate that AceTAC degraders can target and degrade the above-mentioned organelles.

Although AceTAC degraders can target mitochondria for degradation, this strategy still has some shortcomings. At this stage, the N-terminal residue of TOM20 is used as the mitochondrial localization signal. However, this is not only distributed in healthy mitochondria but also in damaged mitochondria, and AceTAC degraders may not distinguish between these two, which may degrade healthy mitochondria during the degradation process. Similar to mitochondria, healthy peroxisomes and ER may also be subjected to the degradation process. Therefore, specific sites of damaged organelles need to be explored to avoid the degradation of healthy organelles.

In the future, AceTAC degraders may be further developed to expand their applications to other fields, such as removing other harmful components in cells, including viruses. Research has shown that autophagy is an important defense mechanism against viruses (Li et al., 2020). As an autophagy-based antibody compound, AceTAC degraders may be able to target the degradation and clearance of certain types of viruses, and thus treat related diseases. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the genus Coronavirus. SARS-CoV-2 encodes 29 proteins, which can lead to coronavirus disease 2019 (COVID-19) (Liu et al., 2023). The latest research indicates that the SARS-CoV-2 ORF8 protein can inhibit the binding of p62 to LC3, which leads to autophagy inhibition, finally promoting p62 accumulation (Tan et al., 2023). Furthermore, ORF8 interacts with p62, forming ORF8/p62 condensation through phase separation, which interferes with the p62 selective autophagy process, further exacerbating autophagy inhibition and ultimately inhibiting viral degradation. AceTAC degraders may act as autophagy receptors like p62 to activate autophagy, which can enhance the clearance of SARS-CoV-2. However, whether AceTAC degraders can promote SARS-CoV-2 degradation by using recognition targets of SARS-CoV-2 to activate autophagy remains to be further established.

Taken together, AceTAC degraders can break down toxic neuroprotein aggregates including mHTT TDP-43 and Tau, thus may serve as a novel strategy for the treatment of NDDs. AceTAC degraders can also target intracellular organelles including mitochondria, peroxisomes, and ER. However, AceTAC degraders in their current development form may not be able to distinguish between healthy and damaged organelles. In the future, the AceTAC degraders may be further modified to expand their applications to other fields, such as breaking down SARS-CoV-2.

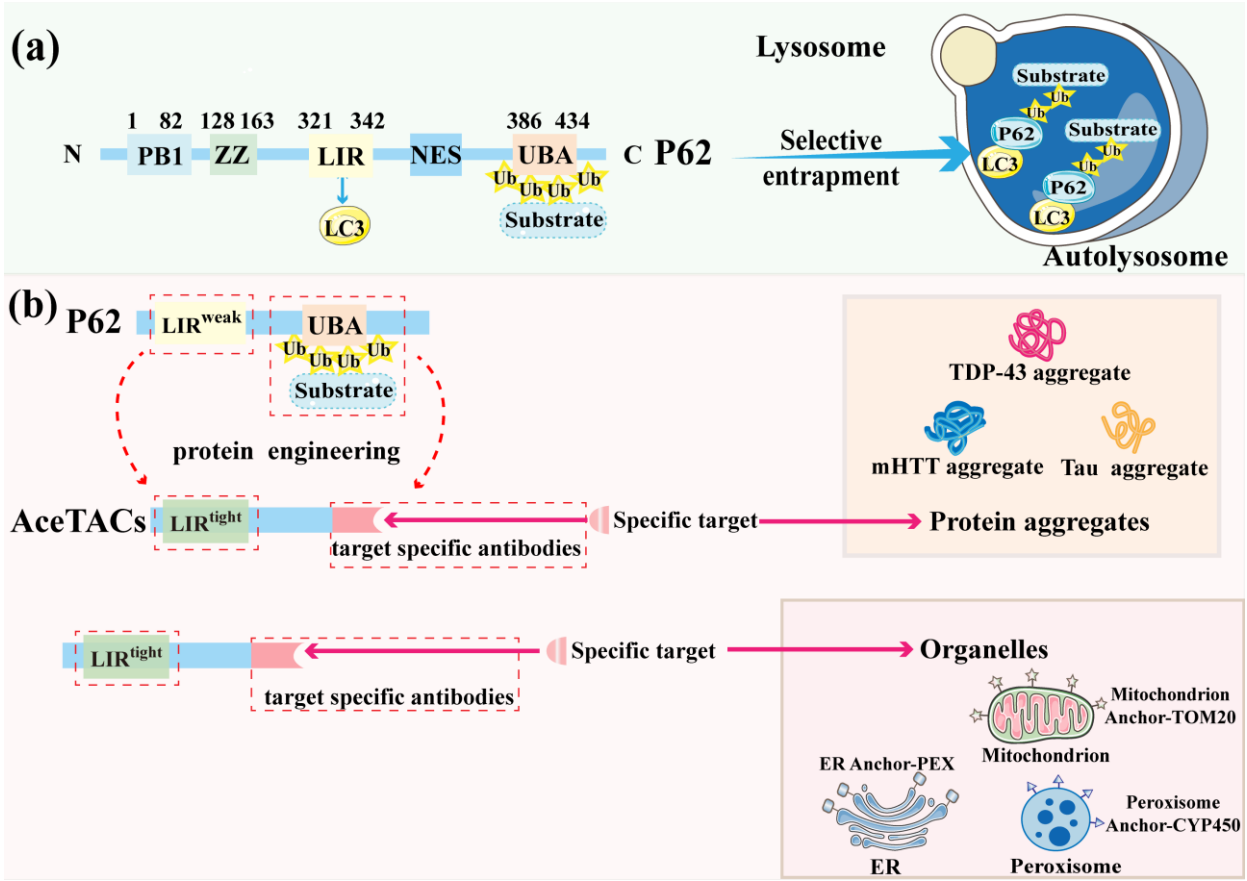

Fig. 1 Structure and function of p62, along with its selective autophagy process, design principle and application of AceTACs degraders. (a) The p62 ubiquitin-associated UBA domain interacts with Ub substrates to form aggregates during selective autophagy. Subsequently, these aggregates are driven into the forming autophagosome through the interaction between the LIR of p62 and LC3 on the autophagosome membrane. (b) AceTAC is suitable for the targeted degradation of various proteins that are prone to aggregation, such as mHTT, TDP-43 and Tau. In organelles, TOM20, PEX3 and CYP450 are identified as recognition sites on the outer membrane of mitochondria, peroxisomes and endoplasmic reticulum, respectively. The AceTAC degrader targets these recognition sites to break down organelles.

Data availability statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Author contributions

Liwen Wang performed wrote and revised the manuscript. Huimei Liu and Lanfang Li both contributed to revise the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Liwen Wang, Huimei Liu, and Lanfang Li declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent

was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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