Targeted protein degradation: mechanisms, strategies and application

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Traditional drug discovery mainly focuses on direct regulation of protein activity. The development and application of protein activity modulators, particularly inhibitors, has been the mainstream in drug development. In recent years, PROTeolysis TArgeting Chimeras (PROTAC) technology has emerged as one of the most promising approaches to remove specific disease-associated proteins by exploiting cells' own destruction machinery. In addition to PROTAC, many different targeted protein degradation (TPD) strategies including, but not limited to, molecular glue, Lysosome-Targeting Chimaeras (LYTAC), and Antibody-based PROTAC (AbTAC), are emerging. These technologies have not only greatly expanded the scope of TPD, but also provided fresh insights into drug discovery. Here, we summarize recent advances of major TPD technologies, discuss their potential applications, and hope to provide a prime for both biologists and chemists who are interested in this vibrant field.

PROTEIN DEGRADATION PATHWAYS: PROTEASOMAL AND LYSOSOMAL PATHWAYS

Protein homeostasis, also known as proteostasis, refers to a highly complex and interconnected process used by cells to maintain concentration, conformation, and subcellular localization of proteins. It comprises a large set of pathways that control protein synthesis, folding, protein transport, and disposal. In eukaryotic cells, damaged proteins or organelles can be cleared by proteasomes or lysosomes. The two pathways are independent but inter-connected with each other. In general, proteasomes eliminate short-lived proteins and soluble misfolded proteins by the ubiquitin–proteasome system (UPS). In contrast, lysosomes are responsible for degradation of long-lived proteins, insoluble protein aggregates, even entire organelles, macromolecular compounds, and intracellular parasites (e.g. certain bacteria) via endocytosis, phagocytosis, or autophagy pathways.

Proteasomes are part of the UPS responsible for degradation of proteins that are damaged, unfolded, and useless. In addition to proteasomes, the UPS also compromises various ubiquitin ligases and de-ubiquitinating enzymes (DUBs). The 76-residue ubiquitin protein is attached to proteins via a lysine isopeptide bond as a post-translational modification (PTM) through sequential reaction involving three enzymes: a Ub activating enzyme (E1), a Ub conjugating enzyme (E2), and a Ub ligase (E3). E1 binds to the ubiquitin molecule in an ATP-dependent manner and then transfers it to E2 via an interaction with E2. Next, E3 catalyzes the transfer of the ubiquitin molecule from E2 to substrates. The repeated action of these three enzymes lead to the polyubiquitination of the substrate. There are eight different polyubiquitin chains (seven lysine residues: K6, K11, K27, K29, K33, K48, K63 and one methionine residue) depending on the residue number of the ubiquitin molecule that is conjugated. Among them, K48 and K63 linkages are the most abundant and account for ~80% of total linkages in mammalian cells. Proteins marked with K48-linked ubiquitin chains are often targeted to proteasome for degradation; in contrast, K63-linked chains do not function in proteasomal degradation, but play a pivotal role in regulating lysosome functions and inflammatory response.

Lysosomes are the primary degradative compartments of the cells, and receive their degradation substances via endocytosis, phagocytosis, or autophagy (Fig. 2). Following endocytosis, some cell surface proteins are recycled to the plasma membrane or other organelles, whereas others are marked with K63-linked ubiquitin chains and sorted into the endosomal sorting complex required for transport (ESCRT) complex degradation pathway. Phagocytosis is a specific form of endocytosis by which cells engulf microbial pathogens or other large particles. Finally, autophagy is an evolutionarily conserved process that cells use to remove unnecessary or dysfunctional intracellular organelles and proteins through a lysosome-dependent manner. Targeted organelles and proteins are wrapped into a double-membrane-bound vesicle, known as autophagosome. The autophagosome then fuses with lysosomes to break down the contents.

Targeted protein degradation (TPD), via the proteasomal and lysosomal pathways, represent a novel tool to explore cellular pathways and a promising therapeutic approach. The concept of TPD was first proposed in 1999 (Fig. 3). Most TPD strategies, such as PROTACs, molecular glues, degradation tags (dTAGs), trim away, and specific and non-genetic inhibitors of apoptosis protein-dependent protein erosive agents (SNIPERs), rely on the UPS and mainly target intracellular proteins. Lysosome-dependent TPD strategies could degrade membrane proteins, extracellular...
Fig. 1  Protein degradation via the ubiquitin-proteasome system (UPS). Proteins undergo ubiquitin-dependent degradation by a suite of three enzymes. E1 interacts with E2, and transfers the ubiquitin molecule to E2. E2 interacts with E3-binding substrate and transfers the ubiquitin molecule to the substrate. Repetition of these processes results in polyubiquitination of the substrate, which is subsequently degraded by the 26S proteasome.

Fig. 2  Protein degradation via three distinct lysosome pathways. (1) Cell surface proteins arrive at endosome after endocytosis. They could be degraded by lysosome, or transported to the plasma membrane or other cellular organelles for recycling. (2) In the phagocytic pathway, cells engulf large extracellular particles, such as invading pathogens and dead cells, and then degrade them by lysosome. (3) Misfolded or aggregated proteins, damaged organelles, and intracellular pathogens, are removed by the autophagy–lysosome pathway. There are three different forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy.
proteins, and protein aggregates, thus greatly expanding the range of substrates. In this review, we first provide a simple introduction of protein degradation mechanisms. We will then summarize recent advances in developing various TPD technologies, and highlight their potential applications in disease treatment. Interested readers are encouraged to read other excellent reviews that cover other aspects of the field.36–41

TARGETED PROTEIN DEGRADATION VIA PROTEASOME

In the canonical ubiquitination pathway, ubiquitin is conjugated to target proteins by an E1-E2-E3 enzymatic cascade (Fig. 1). As the E3 ligase is responsible for recognizing substrates and its family number greatly exceeds E1 and E2, the UPS-based TPD strategies utilize E3 ligases as targeting proteins for degradation.42 PROTAC and molecular glue are two major technologies that rely on the UPS for the degradation of protein of interest (POI), and will be the focus of our discussion (Table 1). Additionally, many PROTAC-based technologies, including selective androgen receptor degrader (SARD),43–45,47 TF-PROTAC,48 dual-PROTAC,49 and selective estrogen receptor degrader (SERD),50–54 have recently emerged (Table 1).

PROTAC

A PROTAC molecule comprises an E3-recruiting ligand, a POI-targeting warhead, and a flexible linker linking the two ligands (Fig. 4a). The addition of PROTAC promotes the formation of the POI-PROTAC-E3 ternary complex, induces ubiquitination of the POI and subsequent degradation via the UPS.37,55,56 Crews and Deshaies groups developed the first PROTAC molecule in 2001.31 The protein-targeting chimeric molecule 1 (Protac-1) was synthesized to recruit target protein methionine aminopeptidase-2 (MetAP-2) to the Skp1-Cullin-F-box (SCF) ubiquitin ligase complex, and subsequently degraded31(Fig. 3). The Protac-1 contains two domains: one domain consists of a phosphopeptide derived from IκBα (IPP) and binds to SCF, and the other domain, composed of ovalicin, interacts with MetAP-2.31 Subsequently, the same group demonstrated that a chimeric molecule consisting of the IκB phosphopeptide and small-molecules could be used to degrade the estrogen receptor (ER) and androgen receptor (AR), which promote the growth of breast and prostate cancers, respectively.57

In 2008, Crews’s group reported the first example of small molecule-based PROTAC58 (Fig. 3). This PROTAC, consisting of nonsteroidal androgen receptor ligand (SARM), a MDM2 ligand targeting ubiquitin ligase murine double minute 2 (MetAP-2) to the Skp1-Cullin-F-box (SCF) ubiquitin ligase complex, and subsequently degraded51(Fig. 3). The Protac-1 contains two domains: one domain consists of a phosphopeptide derived from IκBα (IPP) and binds to SCF, and the other domain, composed of ovalicin, interacts with MetAP-2.31 Subsequently, the same group demonstrated that a chimeric molecule consisting of the IκB phosphopeptide and small-molecules could be used to degrade the estrogen receptor (ER) and androgen receptor (AR), which promote the growth of breast and prostate cancers, respectively.57

In comparison with peptide-based PROTACs, small molecule PROTACs are more readily taken up by cells and more likely to be developed into drugs.59 In addition to MDM2, multiple other E3 ligases have been harnessed in the PROTAC technology, including cereblon (CRBN),60 Von-Hippel-Lindau (VHL),61 and cell inhibitor of apoptosis protein (cIAP).62
PROTACs afford multiple advantages compared with traditional small molecule inhibitors. First, PROTACs greatly expand the range of druggable proteins. More than 4000 disease-associated proteins have been identified. Among them, only ~400 proteins have been successfully exploited in current therapies. Many of them could not be targeted by traditional inhibitors due to their structural complexity, off-target effects and so on. Second, traditional inhibitors only block part of the protein’s function, while PROTACs degrade the protein, thus eliminating all its functions. Third, traditional kinase inhibitors often lead to drug resistance via mutations or overexpression of drug targets, but PROTACs could minimize drug resistance from long-term selection pressure by degrading target proteins. Last, PROTACs are active in a substoichiometric and catalytic manner, which allows them to function at low concentrations, thereby reducing possible toxic side effects.

Molecular glue
Molecular glue facilitates the dimerization or colocalization of two proteins via forming a ternary complex. They can regulate a variety of biological processes, such as transcription, chromatin regulation, protein folding, localization, and degradation. The first examples of molecular glue are cyclosporin A (CsA) and FK506, which are used as immunosuppressants. Mechanistic studies reveal that CsA and FK506 induce the formation of cyclophilin-CsA-Calcineurin and FKBP12-FK506-Calcineurin complexes, respectively, giving rise to the term “molecular glue”. Subsequently, another immunosuppressive agent rapamycin was also discovered as a molecular glue by stabilizing the FKBP12-rapamycin-FRB (mTOR) ternary complex. In addition to immunosuppression, rapamycin and its analogs also exhibit antifungal, antitumor, and antiaging activities.

Molecular glue degraders induce the interaction between a ubiquitin ligase and a POI, leading to POI ubiquitination and subsequent degradation (Fig. 4b). Although both molecular glues and PROTACs harness the UPS for protein degradation, they have several distinctions (Fig. 4). First, PROTACs are heterobifunctional degraders that simultaneously interact with the E3 ligase and the POI; in contrast, molecular glue degraders could interact with only the ligase (more frequently) or the POI, and induce/ stabilize their interactions. Second, molecular glues do not have a linker, making them smaller molecular weight, increased oral bioavailability, and improved cellular permeability, relative to PROTACs. Last, molecular glues are more difficult to design, although rational design strategies are emerging.

Examples of molecular glue degraders include thalidomide, lenalidomide, and pomalidomide. Interestingly, they have been approved by the FDA for the treatment of various types of tumors long before their functional mechanisms were elucidated. Years later, it was discovered that this class of compounds exert antitumor activities by acting as molecular glues (Fig. 3). They induce the interactions between E3 ligase, cereblon, and its transcription factor substrates IKZF1/3, leading to the degradation of IKZF1/3. With more drug-like properties, it is conceivable that molecular glues will receive more attentions from both academia and pharmaceutical industry.

Double-mechanism degrader
Treatment of complicated diseases, such as cancer, often require more than one targets. Yang et al reported that a small molecule, GBD-9, that can target both bruton tyrosine kinase (BTK) and G1 to S phase transition 1 (GSPT1) (Fig. 5). BTK, a tyrosine kinase and a key regulator of the BCR (B-cell receptor) pathway, is up-regulated in a variety of lymphoma cells. GSPT1, a translation termination factor, is involved in regulation of mammalian cell growth. Interestingly, GBD-9 retains the characteristics of both a PROTAC and a molecular glue. The designer balanced the activities of PROTACs and molecular glues by modulating the length of the linker of BTK PROTACs. It appears that GBD-9 acts as a PROTAC to promote the degradation of RTK and, at the same time, as a molecular glue to promote the degradation of GSPT1. As a consequence, GBD-9 displays stronger anti-proliferative effect in multiple cancer cell lines than ibrutinib, a small molecule BTK inhibitor. Future work will be needed to further illustrate the functional mechanism of GBD-9. As both PROTACs and molecular glues have strength and limitation, it will be excited to see more examples that can harness the strength of both strategies.

**TARGETED PROTEIN DEGRADATION VIA lysosomes**

Lyosomes mediate the intracellular degradation of proteins and organelles in three different ways: endocytosis, phagocytosis, or autophagy. Cells bring extracellular material or membrane proteins in via endocytosis. In phagocytosis, cells bind and engulf viruses, bacteria, or other large particles. Autophagy is a highly conserved cellular process in which misfolded or aggregated proteins, damaged organelles, and intracellular pathogens, are removed. There are three forms of autophagic pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). During macroautophagy, dysfunctional proteins or organelles are recognized by autophagy receptors and selectively enclosed in autophagosomes. Autophagosomes are then fuse with lysosomes and their contents are degraded. In microautophagy, lysosomes directly engulf autophagic cargo and...
lead to its degradation.\textsuperscript{77} In CMA, proteins are selected by chaperones, targeted to lysosomes, and directly translocated across the lysosome membrane for degradation. CMA has two unique features. First, CMA degrades only certain proteins, but not organelles. Second, the formation of autophagosomes is unnecessary in CMA.\textsuperscript{78}

With the intensive research in the endosome-lysosome and autophagosome-lysosome degradation pathways, TPD strategies via the lysosomal pathway, such as LYTAC, AbTAC, ATTEC, AUTAC, bispecific aptamer chimeras, and AUTOTAC have emerged in recent years.\textsuperscript{39,79,80} (Fig. 6 and Table 1). In contrast with proteasome-based TPD, which can only degrade certain intracellular proteins, lysosome-based TPD have potential to remove proteins aggregates, damaged excess organelles, membrane, and extracellular proteins.

LYTAC
LYTAC is a novel technique to induce the degradation of extracellular and membrane proteins via the endosome-lysosome pathway.\textsuperscript{81,82} (Fig. 7). As extracellular and membrane proteins comprise 40% of the encoded proteins and are key contributors to neurodegenerative diseases, autoimmune diseases and cancer, LYTAC is a good complement to PROTACs. LYTAC molecules can simultaneously bind the extracellular domain of a membrane protein, or an extracellular protein, and a lysosome-targeting receptor (TLR) residing on the cell surface (Fig. 7). The formation of a ternary complex leads to protein internalization via clathrin-mediated endocytosis, and the POI is subsequently degraded.

The first reported LYTAC molecule is based on cation-independent mannose-6-phosphate receptor (CI-MPR), also known as IGFR2\textsuperscript{81} (Fig. 7). CI-MPRs facilitate intracellular trafficking of lysosomal enzymes, which are modified by N-glycans capped with mannose-6-phosphate (M6P).\textsuperscript{83} Low pH in late endosomes leads to the dissociation of lysosomal enzymes and CI-MPR. Whereas the former is targeted for lysosomal degradation, CI-MPR is transported to the Golgi apparatus and cell surface for recycling.\textsuperscript{83} This natural process is harnessed to generate the first LYTAC molecules, which consist of a small molecule or antibody fused with synthesized a CI-MPR-targeting ligand, poly-M6Pn.\textsuperscript{81} This LYTAC strategy has shown promises in degradation multiple therapeutically relevant proteins. For example, a LYTAC molecule derived by covalently conjugating poly-M6Pn to the EGFR antibody, cetuximab, was shown to specifically degrade EGFR in a variety of cell lines.\textsuperscript{81} In addition, conjugation of poly-M6Pn with anti-PD-L1 antibody led to a significant decrease of PD-L1 at the cell surface.\textsuperscript{81}

Whereas the expression of CI-MPR is ubiquitous, the expression of certain LTRs is tissue specific. Molecules that target tissue-specific LTRs could induce the degradation of target proteins in specific tissues. Asialoglycoprotein receptor (ASGPR) is a liver-specific LTR.\textsuperscript{84,85} The ASGPR-based LYTAC molecule is made by the fusion of antibodies with N-acetylgalactosamine (GalNAc), that target ASGPR\textsuperscript{82} (Fig. 7). Co-culture experiments demonstrate that this LYTAC technology specifically targets cells that express ASGPR.\textsuperscript{82} With initial success of CI-MPR- and ASGPR-based LYTAC, the search for other LTRs is warranted.

Bispecific aptamer chimera
Similar to LYTAC, Bispecific Aptamer Chimera also mediates the degradation of POI via the endosome-lysosome pathway\textsuperscript{79} (Fig. 7). In contrast with LYTAC, Bispecific Aptamer Chimera utilizes DNA aptamer targeting CI-MPR and the transmembrane POI (Fig. 7). The Han team designed the first Bispecific Aptamer Chimera molecule named A1-L-A2, in which A1 and A2 specifically bind to CI-MPR and a POI, and L stands for a linker DNA.\textsuperscript{79} The aptamer chimeras could shuttle membrane proteins, such as receptor tyrosine kinase MET and PTK-7, to lysosomes for degradation.\textsuperscript{79} At the same time, the aptamer chimeras had no significant effect on the levels of non-targeting proteins. Overall, this method provides a powerful, efficient, and versatile platform to induce the degradation of membrane proteins. Nucleic acid aptamers have many advantages relative to antibodies, including simple preparation, precise synthesis, and stability.

AbTAC
Antibody-based PROTAC (AbTAC) is another emerging TPD technology that induces the degradation of extracellular and membrane proteins\textsuperscript{86} (Fig. 8). Compared to conventional PROTAC, AbTAC can target membrane proteins, thus, greatly extending the potential substrates of current TPD strategies. Although bearing the name of PROTAC, AbTAC is more closely related with LYTAC. AbTAC utilizes bispecific antibodies, with one arm targeting a cell-surface POI, and the other arm targeting a transmembrane E3 ligase, such as RNF43\textsuperscript{87} (Fig. 8). The addition of AbTAC molecule induces the complex internalization and subsequent lysosomal degradation of the POI.

Similar to LYTAC, AbTAC also mediate the TPD of cell-surface POI by harnessing the endosome-lysosome pathway (Figs. 6–8). However, the mechanism of action of AbTAC is less clear than that of LYTAC. Particularly, it is unknown whether the intracellular region of a POI is ubiquitinated prior to endocytosis; if it does, how the ubiquitination contribute to the complex internalization. Furthermore, it remains unknown whether RNF43 could be
recycled and re-used like LYTAC receptors, CI-MPR and ASGPR. In addition to RNF43, additional membrane receptors need to be identified for the development of AbTAC technology.

GlueTAC

Recently, another lysosome-based strategy, termed GlueTAC, has been developed to degrade cell-surface proteins (Fig. 9). GlueTAC utilizes three major technologies to facilitate the degradation. First, nanobodies are used to replace conventional antibodies to facilitate cell penetration. Second, covalent interaction is introduced between nanobodies and antigens to overcome relatively low binding affinity and to minimize off-target effects. Third, a cell-penetrating peptide and lysosome-sorting sequence (CPP-LSS) is conjugate to the nanobodies to promote the internalization and lysosomal degradation (Fig. 9). To demonstrate the effectiveness of GlueTAC, the authors developed a GlueTAC molecule targeting PD-L1. This GlueTAC molecule is more effective in reducing the level of PD-L1 in cells and inhibiting tumor growth in immunodeficient mice, in comparison with FDA-approved antibody against PD-L1, Atezolizumab.

Whereas GlueTAC represent another exciting approach in degrade cell-surface proteins, several issues need to be considered. First, the safety. GlueTAC introduces unnatural amino acids in nanobodies and creates covalent bonds between nanobodies and antigens. Thus, the safety of GlueTAC molecules need to be carefully assessed. Second, the nanobodies do not have heavy chains and cannot bind to FcRn. The half-life of GlueTAC also needs to be determined.

AUTAC

In addition to the endosome–lysosome pathway, the autophagy–lysosome pathway provides another avenue for TPD (Fig. 6 and Table 1). Nucleotide 8-nitrocyclic guanosine monophosphate (8-nitro-cGMP) is an important signaling molecule in cells to mediate the recruitment of autophagosomes. This property of 8-nitro-cGMP was used for the development of autophagy-targeting chimera (AUTAC) (Fig. 10). An AUTAC molecule consists three parts: a cGMP-based degradation tag, a linker, and a small molecule ligand for a POI or an organelle. An AUTAC molecule triggers K63-linked polyubiquitin, and...
subsequent lysosome-mediated degradation (Fig. 10). In contrast, a PROTAC molecule induces K48-linked polyubiquitin and proteasome-mediated degradation.

In addition to cytoplasmic proteins, cellular organelles such as mitochondria could be degraded via AUTAC. Mitochondrial dysfunction is associated with many aging-related diseases, and the removal of dysfunctional or damaged mitochondria may ameliorate these diseases. Takahashi et al. developed a molecule known as AUTAC4, which promotes mitophagy of fragmented mitochondria. AUTAC4 utilizes a 2-phenylindole derivative, which is a ligand for a transporter on the outer mitochondrial membrane, as a mitochondria binder. The treatment of AUTAC4 was shown to restore mitochondrial membrane potential and ATP production. These results indicate broad applications of AUTAC, and it is expected to see more interesting applications of AUTAC, such as the degradation of protein aggregates.

**ATTEC**

Similar to the autophagy-based AUTAC, autophagosome tethering compound (ATTEC) functions by tethering the POI to the autophagosome (Figs. 6 and 11). Whereas AUTAC recruits autophagosomes for degradation, ATTEC binds to LC3, one of the key proteins of autophagosome. Lu and coworkers discovered a set of small molecules that are capable of binding to LC3 protein and pathogenic mutant huntingtin proteins. Remarkably, these molecules can distinguish wild-type and mutant huntingtin proteins, which are identical except for the length of the polyglutamine (polyQ) stretch. Mutant huntingtin protein has at least 36 glutamines. The longer the polyQ stretch, the earlier symptoms typically appear. The researchers proposed that these molecules recognize the conformation of the expanded polyQ stretch in the mutant protein and distinguish them from the wild-type protein. By specifically recognizing the mutant...
huntingtin protein, ATTEC provides new possibility for the treatment of Huntington disease. Furthermore, it will be interesting to determine whether these ATTEC molecules can be used for other polyQ diseases, such as dentatorubral pallidoluysian atrophy and Machado-Joseph disease.

Recently, Lu and colleagues further extend the application of ATTEC by developing small molecules targeting Lipid droplets (LD-ATTEC), the fat-storage organelles in cells. These compounds bind LC3 protein as well as Lipid droplets, and can reduce the number of Lipid droplets at micromolar concentrations. Furthermore, they can rescue LD-related phenotypes in two independent mouse models. Collectively, these studies demonstrate that ATTEC could harness the autophagy-lysosome pathway for the degradation of proteins and non-protein materials.

**AUTOTAC**

The autophagy cargo receptor p62/SQSTM1 functions to bridge polyubiquitinated cargo and autophagosomes. Polyubiquitinated
Cargos bind to the UBA domain of p62, leading to a conformational change in p62. Such a conformation change exposes the LIR motif of p62, and promotes its interaction with LC3 on the autophagic membrane. Ji et al. designed the AUTOphagy-Targeting Chimera (AUTOTAC) platform that bypasses the requirement of ubiquitin.

AUTOTAC molecules consist of a module that interacts with the ZZ domain of p62, and a POI-targeting module. The addition of AUTOTAC molecules bridges the POI and p62, independent of ubiquitin on the POI. AUTOTAC promotes the oligomerization and activation of p62, leading to the degradation of the POI by the autophagy–lysosome pathway.

AUTOTAC can mediate the targeted degradation of not only monomeric proteins, but also aggregation prone proteins. Using murine models expressing human pathological tau mutants, Ji et al. demonstrated the AUTOTAC could effectively remove misfolded tau. In contrast, the proteasome-based technologies, such as PROTAC and molecular glue, are usually ineffective in dealing with the misfolded proteins. In addition to Tau, AUTOTACs could also efficiently remove multiple oncoproteins, such as degrading androgen receptor (AR).

CMA-based degrader

In chaperone-mediated autophagy, heat shock protein 70 (HSC70) recognizes soluble protein substrates with KFERQ sequence. The HSC70-substrate complex then binds to lysosomal associated membrane protein 2A (LAMP2) on the lysosomal membrane, and the substrate is then translocated to lysosome lumen for degradation. Thus, a chimera peptide containing the KFERQ sequence and a targeting protein-binding sequence could be exploited to degrade pathogenic or misfolded proteins. CMA-based degraders include three functional domains: a cell membrane penetration sequence, a POI-binding sequence, and a CMA-targeting motif (Fig. 12). Upon the addition to the cells, a CMA-based degrader first enters the cell, then binds to the target protein via the POI-binding sequence, and finally transports to the lysosomes for degradation. Indeed, this strategy has been shown to reduce the levels of mutant huntingtin protein, PSD-95, death-associated protein kinase 1 (DAPK1), as well as α-synuclein.

To become an effective therapeutic strategy, CMA-based degraders need to overcome at least two major hurdles. First, the stability of the degrader. Second, the delivery efficiency. Overall, whereas the CMA-based degraders represent a new approach in TPD, they face great challenges that are not seen by other TPD technologies, such as PROTAC and LYTAC.

APPLICATION OF TARGETED PROTEIN DEGRADATION IN DISEASE TREATMENT

The last few years have seen explosive growth in the field of TPD. Currently, about ten TPD molecules are in the cancer clinical trials. In addition to cancer, many TPD molecules show great promising for the treatment of neurodegenerative diseases, inflammatory diseases, or viral infection.

Cancer

Multiple TPD molecules (mostly based-on the PROTAC technology) have shown potential therapeutic effects in cancer clinical trials and preclinical studies. Due to the space limitation, we can only highlight a few examples. The estrogen receptor (ER) is a master regulator of gene expression, and is critical for the pathogenesis of breast cancer. ARV-471 is a PROTAC molecule developed by Arvinas and specially target ER. In preclinical experiments, ARV-471 leads to efficient ER degradation and significantly reduces tumor burden in xenograft models. Now in phase II clinical trial, ARV-471 can be given either as a single agent or in combination with a CDK4/6 inhibitor. In clinical experiments, ARV-471 shows good oral bioavailability and favorable tolerability.

In addition to ARV-471, ARV-110 is another PROTAC small molecule entering phase II clinical trial. ARV-110 selectively targets androgen receptor (AR) and leads to its degradation. ARV-110 is developed as a potential treatment for prostate cancer, the second most common malignancy in men after lung cancer.
proteins. As a result, novel drug discovery modes, such as TPD, are often based on modulating the functions of target aggregates formed by protein misfolding. Misfolded proteins and Huntington disease (HD), are closely associated with insoluble neurodegenerative diseases (NDs), including Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington disease (HD), are closely associated with insoluble aggregates formed by protein misfolding. Misfolded proteins often display unusual protein-protein interactions (PPIs) that are unrelated with their normal functions. Traditional drug discovery is often based on modulating the functions of target proteins. As a result, novel drug discovery modes, such as TPD, are urgently needed in order to develop therapeutic approaches for NDs.

In 2016, Chen and Li groups reported a PROTAC molecule targeting tau protein, the first attempt to apply the PROTAC technology for the treatment of NDs. The PROTAC molecule they designed is a chimera construct made of a tau-binding peptide, a linker, a VHL-binding peptide, and a cell-penetrating peptide. This molecule leads to a significant degradation of tau and reduced neurotoxicity of AD. Another polypeptide PROTAC for AD was developed by Jiang et al, who used a CRLKep1-binding sequence. This molecule also successfully achieved tau protein degradation.

Inflammatory diseases

In addition to cancer and neurodegenerative diseases, the reach of TPD has extended to inflammatory diseases and immunology. IRAK-4 (interleukin-1 receptor-associated kinase 4) is a member of the IRAK kinase family and involved in Toll-like receptor (TLR) and IL-1R signaling pathways. Upon TLR activation, IRAK-4 is recruited to form the Myddosome complex, which subsequently leading to the phosphorylation of other members of the IRAK family, such as IRAK1 and IRAK2. In addition to its enzymatic activity, the scaffolding role of IRAK-4 in TLR signals is also well established. In comparison with conventional inhibitors, IRAK-4 degraders provide great advantages by eliminating both enzymatic and non-enzymatic functions of IRAK-4. Indeed, multiple IRAK-4-targeting PROTAC molecules have been developed, with one entering phase I clinical trials to treat autoimmune diseases.

BTK is an established target in both inflammation and cancer. Although BTK inhibitors have been proved and used in the clinic to treat different hematological cancers, such as leukemia and lymphoma, the appearance of BTK mutations renders these drugs less effective. These challenges could be uniquely addressed by BTK degraders as these molecules may degrade both wild-type and mutant BTK proteins. Two BTK PROTACs are currently in a phase I trial for the treatment of B cell malignancies and autoimmune diseases.

Viral infection

Viral infection poses a great challenge in global health. SARS-CoV-2 is one of the worst examples, which have infected over 400 million individual and killed 5.7 million worldwide. TPD...
could represent a novel antiviral therapeutic approach. One of
the first successful examples is used for the degradation of hepatitis C
virus (HCV) NS3/4A protease. de Wispelaere et al.146 showed that
telaprevir (the HCV protease inhibitor)-based PROTACs could
inhibit HCV in a cellular infection model. Currently, there are great
interests in the development of PROTACs that target SARS-CoV-2
across academia and industry.157 In addition to PROTAC,
technologies targeting the autophagy–lysosome pathway, such as
AUTAC and ATTEC, could be also used to eliminate key viral
proteins.152

SUMMARY AND OUTLOOK
The past two decades have seen the birth and boom of the TPD
technologies. PROTAC and molecular glue are the most advanced
TPD technology. Both are based on the ubiquitin-proteasome
system and useful for the degradation of intracellular proteins. In
the past 5 years, technologies harnessing the second degradation
pathway in cells have emerged and quickly developed. These
technologies can be further divided into two groups based on their
degradation mechanisms. LYTAC, Bispecific Aptamer Chimeras,
AbTAC, and GlueTAC, degrade extracellular and membrane proteins
by harnessing the endosome-lysosome pathway. In
addition, technologies targeting the autophagy-lysosome pathway,
such as AUTAC, ATTEC, AUTOTAC, and CMA chimeras, can degrade
misfolded proteins, protein aggregation, or damaged organelles.

Multiple PROTAC molecules, including cancer drug candidates
ARV-110 and ARV-471, have shown great promising in clinical
trials. Nevertheless, the PROTAC technology, as a whole, still faces
many challenges. First of all, pharmaceutical properties. PROTAC
molecules often face the challenges of cell permeability and oral
bioavailability due to their large size. Molecular glues are smaller
and have some advantages over PROTAC molecules; however,
they are more difficult to rationally design. Second, the repertory
of E3 ubiquitin ligase. Human genome encodes more than 600 E3
ubiquitin ligases, and only a few of them (VHL, CRBN, IAPs, and
MDM2) have been utilized to degrade target proteins. Third,
toxicity. PROTAC could result in more toxicity than small molecular
inhibitors because they degrade entire targeted proteins, rather
than solely inhibit them.

Relative to PROTAC and molecular glue, the development of
lysosome-based TPD technologies is still in the infancy stage. We still
have much to learn about the specific mechanism of each
technology. As an important intracellular organelle, lysosomes
regulate many important cellular and physiological functions in
addition to protein degradation, such as the metabolism and
homeostasis. It is unclear whether “hijacking” lysosomal degradation
pathway will affect the body as a whole. Expanding the repertory
of lysosome-targeting receptors, which currently include CI-MPR
and ASGPR only, is much needed for LYTAC and similar technologies.
Further characterization of AUTAC, ATTEC, and AUTOTAC molecules,
including systematic study their structure–activity relationship
and understanding of their modes of action, is necessary. These efforts
will help to develop the autophagy-based technologies as a general
method for protein degradation, analogous to PROTAC. Current
CMA-based degraders are mostly limited by cell membrane
permeability and stability, and their small-molecule forms may
overcome these obstacles. Lysosome-based technologies have
greatly broadened the spectrum of targets by PROTAC and
molecular glue, and a surge of interest in this field is definitely
expected. Despite these challenges, TPD technologies, undoubtedly,
will not only provide powerful tools for biomedical research, but
hold great promise for future drug development.

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REFERENCES
1. Anfinsen, C. B. Principles that govern the folding of protein chains. Science 181,
223–230 (1973).
2. Harley, S. E. & Cooper, K. F. Sorting nexins in protein homeostasis. Cells 10, 17
(2020).
3. de Duve, C. The lysosome turns fifty. Nat. Cell Biol. 7, 847–849 (2005).
4. Dikic, I. Proteasomal and autophagic degradation systems. Annu. Rev. Biochem.
86, 193–224 (2017).
5. Wang, X. & Robbins, J. Proteasomal and lysosomal protein degradation and
heart disease. J. Mol. Cell Cardiol. 71, 16–24 (2014).
6. Pohl, C. & Dikic, I. Cellular quality control by the ubiquitin-proteasome system
and the autophagy. Science 366, 818–822 (2019).
7. Kawahata, I. & Fukunaga, K. Degradation of tyrosine hydroxylase by the
ubiquitin-proteasome system in the pathogenesis of parkinson’s disease and
dopa-responsive dystonia. Int. J. Mol. Sci. 21, 3779 (2020).
8. Stern, S. T., Adiseshiaiah, P. P. & Crist, R. M. Autophagy and lysosomal dysfunction
as emerging mechanisms of nanomaterial toxicity. Part Fibre Toxicol. 9, 20 (2012).
9. Ghavami, S. et al. Autophagy and apoptosis dysfunction in neurodegenerative
disorders. Prog. Neurobiol. 112, 24–49 (2014).
10. Martini-Stoica, H., Xu, Y., Ballabio, A. & Zheng, H. The autophagy-lysosomal
pathway in neurodegeneration: a TFEB perspective. Trends Neurosci. 39,
221–234 (2016).
11. Lee, S. et al. PTK2/FAK regulates UPS impairment via SQSTM1/pe2 phosphor-
ylation in TARDBP/TPD-43 proteinopathies. Autophagy 16, 1396–1412 (2020).
12. Ballabio, A. & Bonifacino, J. S. Lysosomes as dynamic regulators of cell and
organismal homeostasis. Nat. Rev. Mol. Cell Biol. 21, 101–118 (2020).
13. Wang, H. et al. HIP1R targets PD-L1 to lysosomal degradation to alter T cell-
mediated cytotoxicity. Nat. Chem. Biol. 15, 42–50 (2019).
14. Majumder, P. & Baumeister, W. Proteasomes: unfoldase-assisted protein
degradation machines. Biol. Chem. 401, 183–199 (2019).
15. Kimura, Y. & Tanaka, K. Regulatory mechanisms involved in the control of ubi-
quitin homeostasis. J. Biochem. 147, 793–798 (2010).
16. Eldridge, A. G. & O’Brien, T. Therapeutic strategies within the ubiquitin protea-
some system. Cell Death Differ. 17, 4–13 (2010).
17. Yuan, T. et al. Inhibition of ubiquitin-specific proteases as a novel anticancer
therapeutic strategy. Front Pharmacol. 9, 1080 (2018).
18. Molineaux, S. M. Molecular pathways: targeting protein-proteasomal degrada-
tion in cancer. Clin. Cancer Res. 18, 15–20 (2012).
19. Tracz, M. & Bialek, W. Beyond K48 and K63: non-canonical protein ubiquitina-
tion. Cell Mol. Biol. Lett. 26, 1 (2021).
20. Ohtake, F. et al. The K48-K63 branched ubiquitin chain regulates NF-kappaB
signaling. Mol. Cell. 64, 251–266 (2016).
21. Yong, X., Billadeau, D. D. & Jia, D. All ways lead to Rome: assembly of retromer
on membranes with different sorting nexins. Signal Transduct. Target Ther. 6,
139 (2021).
22. Yong, X. et al. SNX27-FERM-SNX1 complex structure rationalizes divergent
trafficking pathways by SNX17 and SNX27. Proc. Natl Acad. Sci. USA. 118,
e2105110118 (2021).
23. Cullen, P. J. & Steinberg, F. To degrade or not to degrade: mechanisms and
significance of endocytic recycling. Nat. Rev. Mol. Cell Biol. 19, 679–696 (2018).
24. Mao, L. et al. Phosphorylation of SNX27 by MAPK11/14 links cellular stress-
signaling pathways with endocytic recycling. J. Cell Biol. 220, e202010048
(2021).
25. Lancaster, C. E. et al. Phagocytosis: what’s on the menu? (1). Biochem. Cell Biol.
97, 21–29 (2019).
26. Mizushima, N. & Komatsu, M. Autophagy: renovation of cells and tissues. Cell
147, 728–741 (2011).
27. Mauhe, M. et al. Chloroquine inhibits autophagic flux by decreasing
autophagosome-lysosome fusion. Autophagy 14, 1435–1455 (2018).
28. Nakatogawa, H. Mechanisms governing autophagosome biogenesis. Nat. Rev.
Mol. Cell Biol. 21, 439–458 (2020).
29. Luh, L. M. et al. Frey for the proteasome: targeted protein degradation—a medicinal chemist’s perspective. Angew. Chem. Int. Ed. Engl. 59, 15448–15466 (2020).
30. Gu, S. et al. PROTACs: an emerging targeting technique for protein degradation in drug discovery. Bioessays 40, e1700247 (2018).
31. Sakamoto, K. M. et al. Protacs: chimeric molecules that target proteins to the Skp2–Cul5–F-box complex for ubiquitination and degradation. Proc. Natl Acad. Sci. USA 98, 8554–8559 (2001).
32. Schreiber, S. L. The rise of molecular cells. Cell 184, 3–9 (2021).
33. Nabet, B. et al. The dTAG system for immediate and target-specific protein degradation. Nat. Chem. Biol. 14, 431–441 (2018).
34. Clift, D. et al. A method for the acute and rapid degradation of endogenous proteins. Cell 171, 1662–1706 (2018).
35. Naito, M., Ohoka, N. & Shibata, N. SNIPERs-hijacking IAP activity to induce protein degradation. Drug Discov. Today Technol. 31, 35–42 (2019).
36. Pei, J. et al. Targeting lysosomal degradation pathways: new strategies and techniques for drug discovery. J. Med. Chem. 64, 3493–3507 (2021).
37. Sun, X. et al. PROTACs: great opportunities for academia and industry. Signal Transduct. Target Ther. 4, 64 (2019).
38. Garber, K. The PROTAC gold rush. Nat. Biotechnol. 40, 12–16 (2022).
39. Lin, J. et al. Emerging protein degradation strategies: expanding the scope to extracellular and membrane proteins. Theranostics 11, 8337–8349 (2021).
40. Hu, B. et al. PROTACs: new method to degrade transcription regulating proteins. Eur. J. Med. Chem. 207, 112698 (2020).
41. Liu, J. et al. PROTACs: a novel strategy for cancer therapy. Semin. Cancer Biol. 67, 171–179 (2020).
42. Deng, L. et al. The role of ubiquitination in tumorigenesis and targeted drug discovery. Signal Transduct. Target. Ther. 5, 11 (2020).
43. Bradbury, R. H. et al. Discovery of AZD3514, a small-molecule androgen receptor downregulator for treatment of advanced prostate cancer. Bioorg. Med. Chem. Lett. 23, 1945–1948 (2013).
44. Omlin, A. et al. AZD3514, an oral selective androgen receptor down-regulator in patients with castration-resistant prostate cancer - results of two parallel first-in-human phase I studies. Invest. New Drugs 33, 679–690 (2015).
45. Xie, T. et al. Pharmacological targeting of the pseudokinase Her3. Nat. Chem. Biol. 10, 1006–1012 (2014).
46. Gustafson, J. L. et al. Small-molecule-mediated degradation of the androgen receptor through hydrophobic tagging. Angew. Chem. Int. Ed. Engl. 54, 9659–9662 (2015).
47. Ma, A. et al. Discovery of a first-in-class EZH2 selective degrader. Nat. Chem. Biol. 16, 214–222 (2020).
48. Liu, J. et al. TF–PROTACs enable targeted degradation of transcription factors. J. Am. Chem. Soc. 143, 8902–8910 (2021).
49. Zheng, M. et al. Rational design and synthesis of novel dual PROTACs for simultaneous degradation of EGFR and PARP. J. Med. Chem. 64, 7839–7852 (2021).
50. Jhaveri, K. et al. A phase I study of LSZ102, an oral selective estrogen receptor downregulator with or without ribociclib or alpelisib, in patients with estrogen receptor-positive breast cancer. Clin. Cancer Res. 27, 5760–5770 (2021).
51. McDonnell, D. P., Wardell, S. E. & Norris, J. D. Oral selective estrogen receptor downregulators (SERDs), a breakthrough endocrine therapy for breast cancer. J. Med. Chem. 58, 4883–4887 (2015).
52. Van Knchten, M. et al. Measuring residual estrogen receptor availability during fulvestrant therapy in patients with metastatic breast cancer. Cancer Discov. 5, 72–81 (2015).
53. Zhang, X. et al. Dynamics-based discovery of novel, potent benzoic acid derivatives as orally bioavailable selective estrogen receptor degraders for ErA+ breast cancer. J. Med. Chem. 64, 7575–7595 (2021).
54. Scott, J. S. et al. Discovery of AZD9833, a potent and orally bioavailable selective estrogen receptor degrader and antagonist. J. Med. Chem. 63, 14530–14559 (2020).
55. Wang, Y. et al. Degradation of proteins by PROTACs and other strategies. Acta Pharm. Sin. B 8, 107–208 (2020).
56. Yang, Q., Zhao, J., Chen, D. & Wang, Y. E3 ubiquitin ligases: styles, structures and functions. Mol. Biomed. 2, 23 (2021).
57. Sakamoto, K. M. et al. Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. Mol. Cell. Proteom. 2, 1350–1358 (2003).
58. Schreiber, L. R., Pucheuhat, M., Tae, H. S. & Crews, C. M. Targeted intracellular protein degradation induced by a small molecule: en route to chemical protein-toxins. Bioorg. Med. Chem. Lett. 18, 5904–5908 (2008).
59. Touré, M. & Crews, C. M. Small-molecule PROTACs: new approaches to protein degradation. Angew. Chem. Int. Ed. Engl. 55, 1966–1973 (2016).
60. Wang, C., Zhang, Y., Wu, Y. & Xing, D. Developments of CRBN-based PROTACs as potential therapeutic agents. Eur. J. Med. Chem. 225, 113749 (2021).
122. Heimer, S. et al. Hypertonicity-imposed BCL-XL addiction primes colorectal
Signal Transduction and Targeted Therapy           (2022) 7:113
121. Zhou, H. et al. Structure-based discovery of SD-36 as a potent, selective, and ef
118. Sun, H. et al. Deacetylation of nuclear LC3 drives autophagy initiation under
119. Bai, L. et al. A potent and selective small-molecule degrader of STAT3 achieves
115. Yu, H. et al. Revisiting STAT3 signalling in cancer: new and unexpected biolo-
112. Hanker, A. B., Sudhan, D. R. & Arteaga, C. L. Overcoming endocrine resistance in
104. Gough, N. R., Hatem, C. L. & Fambrough, D. M. The family of LAMP-2 proteins
107. Bekes, M., Langley, D. R. & Crews, C. M. PROTAC targeted protein degraders: the
95. Sawa, T. et al. Protein S-guanylation by the biological signal 8-nitroguanosine
99. Kabeya, Y. et al. LC3, a mammalian homologue of yeast Apg8p, is localized in
98. Huang, R. et al. Deacetylation of nuclear LC3 drives autophagy initiation under
3
compounds.
insights into molecular pathogenesis and therapeutic opportunities. Nat. Rev.
host protection.

93. J. et al. The neural network provides a new strategy to evaluate gavage feeding in
92. Bennett, J. & Starczynowski, D. T. IRAK1 and IRAK4 as emerging therapeutic
targets in hematologic malignancies. Curr. Opin. Hematol. 29, 8–19 (2022).
37. Qin, J. et al. IRAK4 kinase activity is redundant for interleukin-1 (IL-1) receptor-
associated kinase phosphorylation and IL-1 responsiveness. J. Biol. Chem. 279,
26748–26753 (2004).
35. Zhang, J. et al. Assessing IRAK4 functions in ABC DLBCL by IRAK4 kinase inhi-
bition and protein degradation. Cell. Chem. Biol. 27, 1500–1509 e1513 (2020).
34. Chen, Y. et al. Design, synthesis, and biological evaluation of IRAK4-targeting
PROTACs. ACS Med. Chem. Lett. 12, 82–87 (2021).
33. Nunes, J. et al. Targeting IRAK4 for degradation with PROTACs. ACS Med. Chem.
Lett. 10, 1081–1085 (2019).
32. Dobrovolsky, D. et al. Bruton tyrosine kinase degradation as a therapeutic
strategy for cancer. Blood 133, 952–961 (2019).
31. Sun, Y. et al. PROTAC-induced BTK degradation as a novel therapy for mutated
BTK C481S induced ibrutinib-resistant B-cell malignancies. Cell Res. 28, 779–781
(2018).
30. Buhimschi, A. D. et al. Targeting the C481S ibrutinib-resistance mutation in Bruton's Tyrosine Kinase using PROTAC-mediated degradation. Biochemistry 57,
3564–3575 (2018).
29. Wang, A. et al. Low dose of emetine as potential anti-SARS-CoV-2 virus therapy:
preclinical in vitro inhibition and in vivo pharmacokinetic evidences. Mol.
Biomed. 1, 14 (2020).
28. Chen, B. et al. Overview of lethal human coronaviruses. Signal Transduct. Target
Ther. 5, 89 (2020).
27. de Wipelaere, M. et al. Small molecule degraders of the hepatitis C virus pro-
tease reduce susceptibility to resistance mutations. Nat. Commun. 10, 3468
(2019).
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