COMMENTARY

Targeted protein degradation bypassing cereblon and von Hippel-Lindau

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Targeted protein degradation (TPD) has emerged as a new drug discovery approach to wipe out disease-causing proteins by harnessing the ubiquitin-proteasome system (UPS). A major class of molecules in the field are called proteolysis-targeting chimeras (PROTACs), which are heterobifunctional molecules encompassing two ligands that bind to a protein of interest and the E3 ligase, respectively joint by a linker.[1] Due to its catalytic mechanism that enables event-driven pharmacology, PROTACs technology can induce an efficient degradation and therefore achieve the pharmacology at a lower dose compared with traditional small molecule inhibitors that exert their inhibition through an occupancy-driven mechanism. Since the first PROTAC was reported as a proof-of-concept 20 years ago, this technology has led to a paradigm shift that culminated in the clinic trials of new modalities such as ARV-110, ARV-471, etc.[2] However, the PROTACs molecules developed to date have been largely limited to certain E3s including cereblon (CRBN), von Hippel-Lindau (VHL), murine double minute 2 (MDM2), and inhibitor of apoptosis (IAP) proteins. In human proteome, there are more than 600 ubiquitin E3 ligases within three different categories, which leaves a wide-open space to discover ligands for unexplored E3 ligases or other components in the UPS. Expanding the toolbox with new ligases will provide multilayer benefits to TPD such as overcoming or preventing PROTAC resistance due to functional hotspots mutation in CRBN/VHL.[3] In this commentary, we mainly focus on recent efforts on hijacking other natural UPS components including ubiquitin-conjugating enzymes (E2s), new E3 ligases beyond CRBN, VHL, MDM2, and IAPs, and proteasome subunits for TPD (Figure 1).

TARGETED DEGRADATION VIA DIRECT E2 RECRUITMENT

In eukaryotes, there are only around 40 ubiquitin-conjugating enzymes (E2s) that can couple with several E3s. Most E2s are small proteins of ~150 amino acids with conserved ubiquitin conjugation (UBC) domain harboring a catalytic cysteine that can form a thioester bond with ubiquitin. Very recently, Nomura group from University of California, Berkeley, identified a covalent molecular glue degrader EN450 that can target Cys111 of ubiquitin-conjugating enzyme E2D (UBE2D) while sparing Cys85, a catalytic cysteine coupled with the Cullin E3 ligase complex during ubiquitin transfer. Furthermore, they developed NF142 by conjugating EN450 with BET family protein inhibitor JQ1 and demonstrated the degradation of a short isoform of BRD4 in HEK293T cells. Similarly, through activity-based protein profiling (ABPP) screening against recombinant UBE2D2 C85S protein, a new hit EN67 containing an acrylamide warhead was discovered. With EN67 as a UBE2D recruiter, they made NF90 and NF500C as efficient BRD4 and AR degraders. Those efforts are pioneering a new era of PROTAC development that builds diversified modalities by hijacking E2.

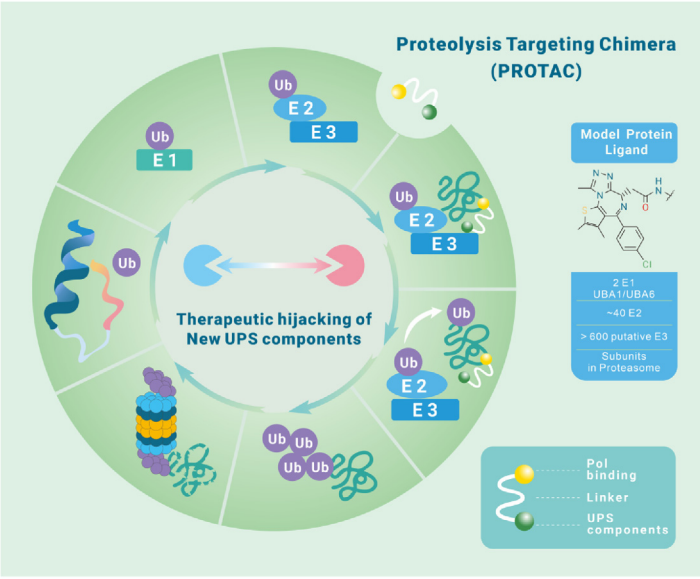

Figure 1. The ubiquitin-proteasome pathway that can be hijacked for PROTAC application BRD4 is the most heavily investigated model protein in early proof-of-concept TPD studies co-opting UPS components beyond CRBN/VHL.

TARGETED DEGRADATION VIA DIRECT NOVEL E3 RECRUITMENT

Tremendous efforts have also been devoted to exploiting alternative tissue-specific E3 ligases by ABPP-based chemoproteomics strategies that can be hijacked for TPD applications.[4] Cravatt lab used isoTOP-ABPP to map the intracellular targets of structurally varied electrophilic groups, which led to the identification of covalent PROTACs through engagement with one or multiple cysteines of DCAF family proteins such as DCAF11 (C443, C460, and C485), DCAF16 (C177 and/or C179), and DCAF1 (C1113). The E3 ligase binders have also been identified by the forward genetic strategy. Through screening resistant clones with mutations that render cells resistant to the toxin, Han et al. found mutation of RBM39 can abrogate the cell-killing effect of a series of sulfonamides compounds such as indisulam. Further mechanistic and structural studies revealed that sulfonamides can act as a molecular glue recruiting CUL4-DCAF15 E3 ubiquitin ligase to degrade RBM39, which successfully translates to the development of DCAF15-based BRD4 PROTACs. Besides DCAF family proteins, Nomura lab from University of California, Berkeley, also identified a series of natural products and synthetic molecules as covalent E3 binders by target/cell-based covalent fragment screening including nimbolide (RNF114-C8), EN219 (RNF114-C8), TRH 1–23 (RNF4-C132/135), and EN106 (FEM1B-C186).

There are also some efforts that harness other E3 ligases employing non-covalent binders to expand the arsenal for TPD. In 2019, Ohoka et al. used β-naphthoflavone (β-NF) or ITE as the recruiter for the aryl hydrocarbon receptor (AhR) E3 ligase and developed chimeric degrader molecules that can induce the degradation of cellular retinoic acid binding proteins and BRD family proteins. Very recently, Jin lab from Icahn school of medicine at Mount Sinai and Gray lab from Stanford University developed Kelch-like ECH-associated protein 1 (KEAP1)-based PROTACs utilizing KI696 as the KEAP1 binder, which led to the successful development of BRD4 or murine focal adhesion kinase degraders. In 2022, Farrell et al. designed a macrocyclic molecule BTR2000 engaging KLHL20 based on KLHL20 substrate DAPK1. BTR2000-derived PROTAC molecule BTR2003 successfully degraded BET family proteins in cancer cells, which demonstrates the feasibility to design ligands derived from natural E3 substrates. Encouragingly, drug discovery targeting E3 ligases is a burgeoning field, and those motivative efforts yielded a series of chemical probes targeting E3s that have never been drugged before such as suppressor of cytokine signaling 2 (SOCS2) and the human CTLH E3 ligase subunit glucose-induced degradation protein 4 homolog (GID4), which expedite PROTAC development leveraging those chemical handles in the future.

TARGETED DEGRADATION VIA DIRECT PROTEASOME RECRUITMENT

The 26S proteasome, a 2.5-MDa proteolytic machine, is composed of two 19S regulatory particles (RPs) that process ubiquitinated substrates and a cylindrical 20S core particle that forms the internal degradation chamber. In 2013, RA190 was identified as a covalent inhibitor targeting Cys88 located at the Pru domain

of ubiquitin receptor RPN13 within the 19S RP of the proteasome. The binding of RA190 seems not able to displace RPN13 from the proteasome, which may spur research interest using RA190 as the starting point to recruit the proteasome for TPD. However, the potential of leveraging RA190 as a proteasome recruiter to deliver ubiquitin and degrade other model proteins is yet to be explored. Although current Rpn13 modulators are not optimal and there are some controversial findings arguing about the selectivity of this compound, mounting evidence collectively demonstrated the Rpn13-Cys88 is at least chemically accessible by covalent modalities and will spur further efforts on identification of a more potent and selective ligand for Rpn13.

Very recently, researchers from Genentech identified peptidic ligand MC1 and its derivatives as the binders of 26S proteasome subunit Rpn1/PSMD2 with single-digit nanomolar binding affinity. The complex structure PSMD2-MC1-Fab was further determined by cryo-EM with a resolution around 2.5 Å. Conjugation of the ligand MC1 to BRD4 ligand with PEG linkers led to the development of chemical inducers of degradation that can robustly degrade BRD4 in HEK293 cells with a DC_{50} value of 0.73 μM. This demonstrates that targeted degradation via direct 26S proteasome recruitment is strategically feasible. Further studies on which transporter might participate in the cellular uptake of those high-molecular weight chemotypes and whether a low-molecular weight compound with a better permeability could be developed will be worthwhile.

OUTLOOK

TPD is a new frontier that offers unprecedented therapeutic opportunities to target undruggable proteins that reshapes the space for drug discovery. In the recent decade, the TPD field has witnessed great success due to the efforts from the whole TPD community. Fueled by ABPP chemoproteomics platform and advanced technology in crystallography and cryo-EM, more structural information is available, which unprecedently facilitates novel drug discovery. Although many small molecules targeting UPS components have successfully been developed for TPD, there are still some caveats. First, most of the proof-of-concept studies are using BRD4 as the model protein, which seems sensitive to degradation in most cases. Thus, the results should be carefully interpreted, and more investigation on non-BET proteins is needed to explore the versatility for protein degradation. Second, most of the current binders of UPS components are unoptimized. There are still persistent challenges to identify selective ligands for novel E3 ligases. Covalent fragment screening in a proteome-wide scale has significantly expanded the repertoire of targetable E3s and yielded electrophilic PROTACs with remarkable degradability and physical chemical properties due to the reduced size of covalent fragments. However, it is noteworthy that most covalent hits have irreversible warheads that lose catalytic advantages and display only modest target engagement in cells with weak reversible binding affinity.[5] More medicinal chemistry efforts will be needed to optimize the hits into more potent and selective ligands for these E3 ligases, and exploration on reversible covalent chemistry may help solve the problem of insufficient catalytic efficiency for covalent PROTACs. Besides, the hits that bind to the intrinsically disordered region and induce the protein folding will require further mechanistic study to provide some rationale for chemistry optimization. Third, most degraders were characterized in limited cell lines. Since PROTAC-induced degradation could be cell line dependent, developing new methods to expand the cell degradation screening to comprehensively understand degrader performance is of utmost importance. With more and more proof-of-concept studies disclosed, we believe PROTACs that recruit new UPS components will fill the gap due to the limitation of current E3 ligases and offer more opportunities for drug discovery.

REFERENCES

1. Chamberlain, P.P., and Hamann, L.G. (2019). Development of targeted protein degradation therapeutics. Nat. Chem. Biol. ***15***, 937–944.
2. Békés, M., Langley, D.R., and Crews, C.M. (2022). PROTAC targeted protein degraders: the past is prologue. Nat. Rev. Drug Discov. ***21***, 181–200.
3. Hanzl, A., Casement, R., Imrichova, H., et al. (2022). Functional E3 ligase hotspots and resistance mechanisms to small-molecule degraders. Nat. Chem. Biol. ***19***, 323–333.
4. Lu, W., Kostic, M., Zhang, T., et al. (2021). Fragment-based covalent ligand discovery. RSC Chem. Biol. ***2***, 354–367.
5. Lu, D., Yu, X., Lin, H., et al. (2022). Applications of covalent chemistry in targeted protein degradation. Chem. Soc. Rev. ***51***, 9243–9261.

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DECLARATION OF INTERESTS

The author declares no competing interests.