RESEARCH HIGHLIGHT

Life mimics art

Muthukumaran Venkatachalapathy[1] and Craig M. Crews[1,2,3]✉

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Ichikawa et al. have discovered that C-terminal cyclic imides imitate the chemical motif of molecular glue degrader thalidomide and act as a naturally occurring degron of the E3 ligase cereblon.

Thalidomide and its derivatives lenalidomide and pomalidomide are immunomodulatory drugs (IMiDs) used in the frontline treatment of hematological malignancies.[1] These IMiDs — particularly the bestselling drug lenalidomide (~$12.8 billion in 2021) — are clinically effective against multiple myeloma, myelodysplastic syndrome, lymphoma, and follicular lymphoma. However, half a century ago, thalidomide treatment resulted in severe birth defects in thousands of babies.[2] By 2010, scientists discovered that thalidomide curbs limb development in zebrafish and chickens through its interaction with cereblon,[3] a evolutionally conserved protein named after its presumed role in cerebral development[4] and whose dysfunction is associated with mild intellectual disability.[5]

Cereblon is the substrate receptor for a CRL4-type E3 ligase complex and acts as a crucial protein for efficient disposal of selected proteins[6] by decorating those native substrates with ubiquitin tags and thereby directing them to the endogenous protein degradation machinery. Thalidomide and its derivatives act like molecular glues: these IMiD drugs induce closer proximity between cereblon and one of many non-native protein substrates. This interaction results in the addition of ubiquitin tags to these neosubstrates. Similar to the native cereblon substate proteins, these ubiquitinated non-native substrates are also degraded by proteasome machinery.[7] Examples of IMiD-induced cereblon neosubstrates include the transcription factors IKZF1/3, SALL4, ZFP91, ZNF692, and the kinase CSNK1A.[8]

A degron is a minimal structural element within a protein that is sufficient for targeting the protein for degradation via the Ubiquitin/Proteasome System (UPS). While some have speculated that IMiDs might mimic a naturally occurring degron, until recently the structural motif recognized by cereblon had not been identified. A recent groundbreaking article published in *Nature* postulated that thalidomide and its derivatives could imitate post-translational modifications (PTMs) such as C-terminal cyclic imides (Fig. 1).[9] They highlighted that cyclization of glutamine or asparagine at the C-terminus of a protein might arise either through the action of enzymes or spontaneous cyclization during protein ageing or specific protein splicing events. Thus, to identify the minimum structural degron motif, the authors designed several ligands mimicking thalidomide that could functionally engage cereblon in cells. As a positive control for cereblon engagement assay, they used the previously reported bromodomain-containing protein 4 (BRD4) inhibitor JQ1 functionalized with thalidomide (dBET6).[10] dBET6 is known to cause the degradation of BRD4 by recruiting it to cereblon.

Biomimetic dipeptides resembling thalidomide structure were linked to JQ1 and subjected to assays that measure cereblon engagement and degradation of BRD4 in cells. As a result, dipeptides with a C-terminal cyclized glutarimide or aspartimide preceded by non-polar or aromatic amino acids were found to induce BRD4 degradation in cells and act as a substitute for thalidomide. The authors confirm that these biomimetic dipeptide-based degraders recruit cereblon and form a ternary complex with BRD4, which was validated using NanoBRET and photo-affinity labeling displacement assays. Furthermore, molecular modeling experiments reveal that like thalidomide, dipeptides with C-terminal imides may mediate interactions with distinct substrates. Interestingly, engineering of GFP to generate C-terminal cyclic imides resulted in its rapid, cereblon-dependent ubiquitination and degradation of GFP in cells, demonstrating that this PTM acts as a degron. Treatment of cells with lenalidomide as a competitor, or replacement of cyclic imides with acyclic ones reduces GFP degradation, validating that these dipeptides are cereblon ligands. Depletion of GFP proteins possessing C-terminal cyclic imides is detected in multiple cell lines and species suggesting that the recognition of the degron by cereblon is conserved. C-terminal imides installed in other proteins such as FKBP12 show similar cereblon-dependent degradation. These results highlight that C-terminal cyclic imide is a transferable chemical motif that acts as a degron recognized by cereblon to promote the targeted protein degradation in cells.

Global proteomics datasets from the NCI7 cell line panel and six primary human tissues revealed the prevalence of this PTM, with thousands of unique cyclic imides (cN or cQ) detected across the proteome. These PTMs are observed across proteins in human tissues, constituting a map that signifies their prevalence that is higher in the human proteome than previously thought. C-terminal cyclic imides ($t_{1/2}$ for cQ, 18.4 h; $t_{1/2}$ for cN, 16.7 h; $t_{1/2}$ for GFP-FcQ, 24.9 h; and $t_{1/2}$ for GFP-FcN, 23.0 h) are relatively stable and have a degradation timeframe comparable to known substrates of cereblon induced by the IMiDs. The authors identified C-terminal cyclic imides in haemoglobin subunits HBA and HBB. Haemoglobin is abundant in red blood cells and has a half-life of ~120 days. The stability of these proteins could possibly arise due to the absence of cereblon in these types of cells. To verify the presence of C-terminal cyclic imides in other endogenous proteins, experiments were carried out in recombinant haemoglobin protein and patient blood samples. Digestion of

[1]Department of Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT, USA. [2]Department of Pharmacology, Yale University, New Haven, CT, USA. [3]Department of Chemistry, Yale University, New Haven, CT, USA. ✉email: craig.crews@yale.edu

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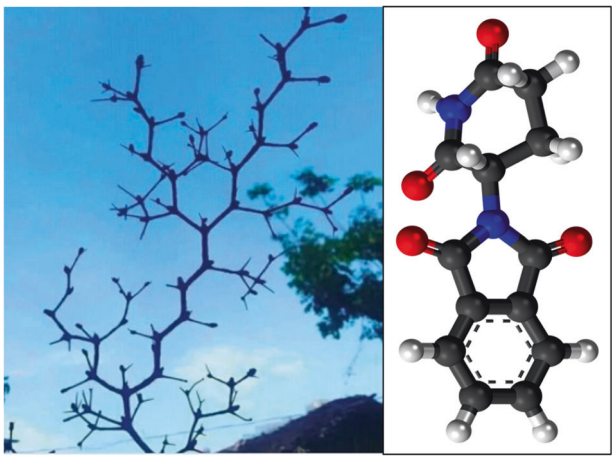

Fig. 1 Branches of a tree resemble a chemical structure highlighting how a plant imitates synthetic art (left). A man-made drug 'thalidomide' developed in the 1950s structurally mimics C-terminal imides which are recently identified as a natural degron of cereblon in 2022 (right).

recombinant haemoglobin gave six cQ and cN sites and digestion of two healthy donor blood samples revealed three unique peptides bearing C-terminal cN modification. This clearly demonstrates the presence of cN and cQ modifications in human samples.

In summary, Christina Woo and colleagues have discovered 'C-terminal cyclic imide' as a minimum structural motif that represents a native cereblon degron. C-terminal cyclic imides resulting from intramolecular cyclization of glutamine or asparagine residues are largely underappreciated protein modifications. Moreover, the groundbreaking work from Prof. Woo's lab will aid in exploring the physiological functions of cereblon and designing better molecular glues with therapeutic value.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Craig M. Crews.