Novel Mechanisms of Molecular Glue-Induced Protein Degradation

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Molecular glues, as defined thus far in the targeted protein degradation (TPD) field, are monovalent small molecules (<500 Da) that reshape the surface of an E3 ligase receptor, promoting the recruitment of neosubstrates and facilitating their ubiquitination and subsequent degradation via the proteasome. The most established molecular glues in TPD are a) the immunomodulatory "IMiD" class of small molecules that bind the E3 ligase cereblon and b) the aryl sufonamides that engage DCAF15. As these molecular glues do not require a druggable pocket on the protein of interest, they are poised to allow targeting of difficult-to-drug proteins. With a catalytic mechanism of action and the ability to target nonenzymatic proteins, molecular glues have exceptional promise as a therapeutic modality.[1] Thus far, molecular glue degraders in TPD have relied on recruiting an E3 ligase receptor; however, three recent studies push the envelope of how molecular glues can be utilized to induce the degradation of neosubstrates.

To discover drugs with an unrealized ability to induce degradation, Słabicki et al. studied the correlation between preclinical and clinical drugs whose cytotoxic effect necessitate the components of the E3 ligase machinery.[2] Through this exercise, they identified CR8, a pan-CDK inhibitor, whose cytotoxicity is dependent on damage-specific DNA binding protein 1 (DDB1) and induces selective degradation of cyclin K. DDB1 is an adaptor protein that coordinates the interaction between the E3 ligase substrate receptor and the CUL4A/B-RBX1 ligase core (Figure 1A, middle). Through extensive profiling using functional genomics, they identify further components of the DDB1 complex (CUL4B, NEDD8, UBEA3, and RBX1) as required for CR8 induced degradation of cyclin K. However, they could not identify an E3 ligase substrate receptor assumed to be crucial for substrate ubiquitination. Rather, they discovered CDK12, an E3 ligase unrelated component, but a known binder of CR8 as required for cyclin K degradation. Using pull-down and TR-FRET studies, they show that CDK12 has a very weak affinity for DDB1, which is strengthened by 500−1000-fold in the presence of CR8. They solved a 3.5 Å structure that confirmed the DDB1-CR8-CDK12 complex revealing a substantial 2100 Å protein−protein interface with CR8 bridging the interaction. Their structure shows that the terminal phenylpyridine ring system engages the beta-propeller C domain of DDB1, which positions cyclin K in the ubiquitination zone of the ligase normally occupied by degraded substrates (Figure 1A, bottom). Interestingly, this result was also observed by two independent groups with unique chemistry,[1,3] further highlighting those molecular glues can bypass the requirement of

an E3 ligase receptor to hijack its function and induce degradation.

A second study from Słabicki et al. established induced polymerization as another mechanism by which molecular glues can induce degradation.[4] In a screen for inhibitors of factor B cell lymphoma 6 (BCL6), they confirmed a previously identified BCL6 inhibitor, BI-3802, unexpectedly induced BCL6 degradation, while a close analog did not. Hints of BI-3802 induced polymerization were established through fluorescence microscopy studies in cells expressing eGFP-BCL-6 where treatment with BI-3802 caused eGFP foci within 10 min of treatment to disappear through degradation within 100 min. In addition, size-exclusion chromatography in the presence of BI-3802 caused higher molecular weight species, while the close analog did not. Remarkably, they find that purified BCL6 in the presence of BI-3802 causes filamentous structures with a sinusoidal shape under negative stain electron microscopy. A cryo-EM structure (3.7 Å) showed that the glue binds a groove between dimers of the Bric-à-brac (BTB) domain of BCL6 and promotes polymerization primarily through hydrophobic interactions (Figure 1B). A slight modification of the solvent exposed group on the analog discouraged polymerization, explaining its inability to induce BCL6 degradation. They find that the E3 ligase, SIAH1, recognizes the BCL6 polymer and promotes its ubiquitination and degradation. Immunoprecipitation studies show that SIAH1 interacts with BCL6 in the absence of BI-3802, but their interaction is greatly enhanced in the presence of the molecular glue. Thus, through polymerization, BI-3802 increases its association with the cognate E3 ligase of BCL6, promoting its degradation.

In a third and more directed approach, Li et al. used microarray screening to identify small molecules that selectively induce the interaction of mutant huntingtin protein (mHTT) and LC3, a protein important in autophagy substrate selection (Figure 1C).[5] While the proteasome is adept at degrading most proteins, it inefficiently recognizes bulky and aggregated substrates. Thus, degradation via autophagy was a more suitable approach for this aggregated target. Their screen

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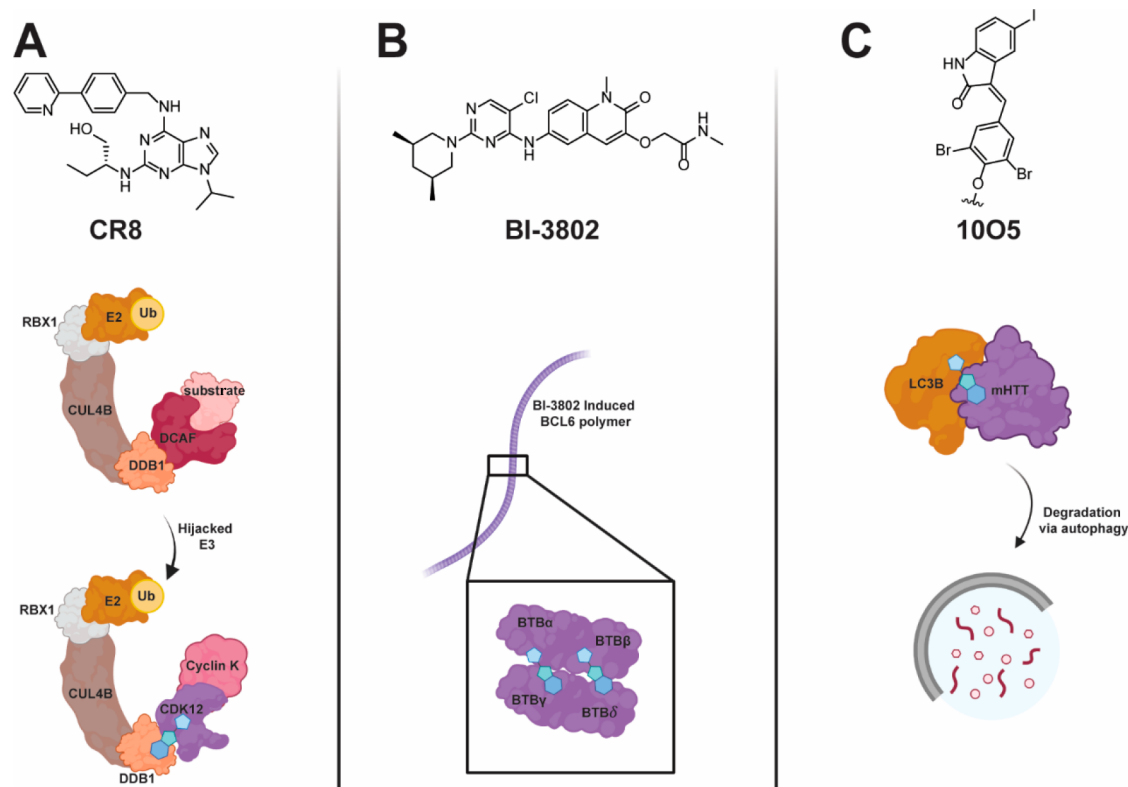
Figure 1. Mechanisms of molecular glue induced degradation that bypass recruitment of an E3 ligase receptor. (A) CR8 hijacks the E3 ligase adaptor, DDB1, as opposed to the E3 receptor, to position cyclin K in the ubiquitination zone, promoting its proteasomal degradation. (B) BI-3802 induces polymerization of the BCL-6 BTB domain. Polymerization enhances recruitment of its cognate E3 ligase, promoting its degradation. (C) ATTECs such as 10O5 link LC-3 to mHTT, promoting its association with elongating phagophores, leading to degradation through autophagy.

identified 2 compounds (10O5, 8F20) that selectively induced degradation of mHTT and not WT Huntington protein, which they termed autophagy tethering compounds (ATTECs). ATTECs achieve selectivity by engaging a 72 glutamine polyQ region that is absent in the WT allele. In cells, the compounds localize to LC3B positive autophagosomes and induce degradation of mHTT in primary cultured neocortical neurons and drosophila models. Furthermore, inhibition of important autophagy mediators abrogated the function of the ATTECs, confirming its mechanism of action. Interestingly, the authors observed a hook effect, a phenomenon associated with heterobifunctional PROTACs explained by the dominance of binary (LC3:ATTEC or ATTEC:mHTT) rather than ternary (LC3:ATTEC:mHTT) protein−drug complexes at higher concentrations of the compound. Thus, it appears that ATTECs, due to their size and behavior, form a unique category that lies between traditional molecular glues (<500 Da) and PROTACS (independently engaging both target and E3 ligase). However, there is a need for more biophysical and structural studies to further solidify the proposed mechanism.

These three studies reveal novel mechanisms by which molecular glues may be utilized to induce degradation of a given protein target, further diversifying the possibilities for drug development. The discovery of CR8 shows that the CRL4 E3 ligase complex can be more readily reprogrammed than previously anticipated; other E3 ligase components beyond the E3 substrate receptor may be co-opted to position target proteins in the respective ubiquitination zone. This study also creatively highlights that the drug target does not have to be the target of ubiquitination. The discoveries with BI-3802 demonstrate that induced polymerization, bearing resemblance

to polymerization or aggregation observed in proteinopathies such as amyloidosis, may be a viable route to allow recruitment of a protein's cognate E3 ligase and cause its degradation. In this example, the ability to "glue" is achieved via solvent-exposed, hydrophobic groups. Small chemical modifications of the solvent exposed region or mutations of the protein interface can completely abrogate degradation, highlighting the finicky nature of molecular glues. On one hand, this serves as a huge benefit that can lead to highly selective drugs, but on the other hand, it makes the design and identification of molecular glues extremely difficult. Indeed, thus far molecular glue identification has relied primarily on serendipity, but a a shift toward rational design is crucial to establish their general utility. Early examples are beginning to emerge, which rationalize some of these concepts to develop molecular glue degraders with new functions, but there is still much to be learned. Small molecule screening approaches such as those used in the identification of ATTECs, tied with thorough structural studies, will certainly expedite the rational discovery of new molecular glues that employ unique mechanisms for induced degradation of novel targets.[1]

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Notes

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