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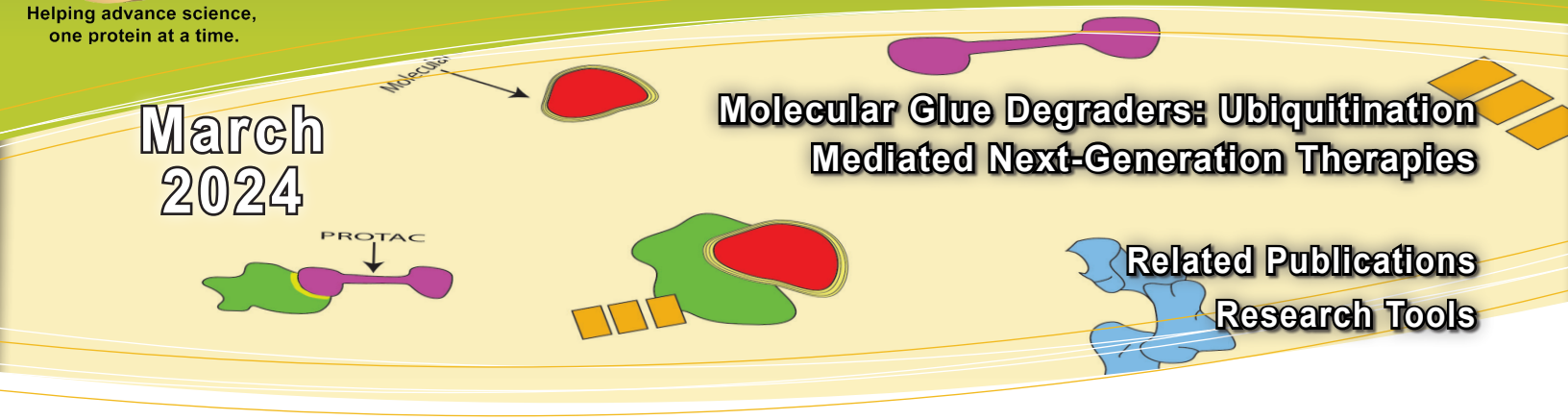

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Molecular Glue Degraders: Ubiquitination Mediated Next-Generation Therapies

Introduction

Targeted protein degradation is an emerging drug discovery strategy that uses small molecules to target disease-causing proteins for degradation mainly via the ubiquitin–proteasome (UPS) pathway. The promise of these drugs over classical small molecule inhibitors is their potential to target "non-druggable" targets, overcome drug resistance, and produce a higher activity or range of action; thus, drugs that target protein degradation are emerging as novel therapeutic approaches in a wide range of diseases including inflammatory disorders, cancers, neurodegenerative diseases, and others[reviewed in 1]. The two prototypical drugs that induce protein degradation include proteolysis-targeting chimera (PROTACs) and molecular glue degraders (MGDs) both of which have shown tremendous promise at targeting aberrant proteins for degradation (see Figure 1). MGDs a class of small molecules that induce, stabilize, or enhance protein-protein interactions between key regulatory proteins and ubiquitin ligases, have exploded in popularity since the identification that the immunomodulatory drug, thalidomide, functions through targeted protein degradation mechanisms[2, 3]. This newsletter will delve into MGDs and emerging targets and therapies.

Overview of Molecular Glue Degraders:

Proper mammalian cellular function requires precise spatiotemporal control of key proteins and molecules; dysfunction of these protein levels disrupts cellular processes like cell cycle progression, migration, differentiation, and even cell death processes like apoptosis. Ubiquitination is a key regulatory process to control appropriate protein levels, as it marks unneeded proteins for degradation via the proteasome[reviewed in 4]. More recently, the properties of the UPS system have been capitalized upon by researchers to target disease-inducing proteins for degradation as a mechanism to treat the disease. Drug-targeting molecular degraders have been around since the early 2000s, first in the form of PROTACs which are heterobifunctional small molecules that link together a ligand targeting a protein of interest and an E3 ligase targeting molecule[reviewed in 1], which was introduced by Deshaies and colleagues[5]. In 2007, Tan X et al. presented the concept of MGDs when they identified that auxin, a critically important plant hormone, could promote the degradation of a family of key transcription repressors by enhancing their otherwise weak interactions with the SCF^{TIR1} ubiquitin ligase complex[6]. Auxin fills a gap between the E3 ligase and the degron motif of its substrate, which is the same mechanism of action utilized by immunomodulatory drugs (IMiDs) like thalidomide to promote the ubiquitin-dependent degradation of a host of cellular proteins [reviewed in 1]. In the case of IMiDs, this class of drugs enhances the protein-protein interactions between its targets and cereblon (CRBN), the substrate receptor Cullin Ring E3 ubiquitin ligase 4 (CRL4) complex IMiDs are now widely used in the clinic to treat multiple myelomas and other B cell malignancies. Due to the success of IMiDs, researchers have sought to capitalize on this drug modality through the development of synthetic MGDs that target neosubstrates. Interestingly, this target protein degradation mechanism has also been revealed for antitumor aryl-sulfonamides, which reprograms another CRL4 substrate receptor, DCAF15, to bind and degrade certain RNA-binding proteins [reviewed in 1].

Systematic Screening Approaches – Identification of CDK12-Cyclin K Degraders

Identification of novel MGs has been elusive for a multitude of reasons and has primarily occurred serendipitously like in the cases of Auxin and IMiDs. However novel work by the Ebert group led to the identification of a CDK12-cyclin K MGD, which they called CR8[6]. In the study, they utilized a systematic approach to identify correlations between the cytotoxicity of over 4,000 small molecules and E3 ligase expression in hundreds of cancer cell lines to identify the small molecule CR8 as a compound that acts as a MGD[6]. The group went on to show that a solvent-exposed pyridyl moiety of CR8, induces CDK12-cyclin K complex formation with DDB1, which led to cyclin K ubiquitination and degradation[6]. In another study, high throughput screening was used to identify the small molecule HQ461 which promotes the interaction of CDK12 and the DDB1-CUL4-RBX1 E3 ubiquitin ligase[7], which led to a similar

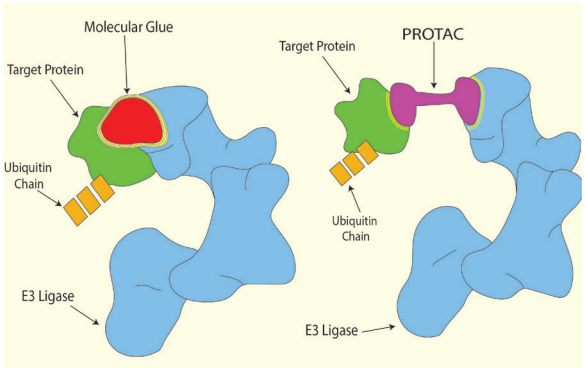

Figure 1. Schematic showing differences in the mechanism by which molecular glues interact with substrate to promote E3-ligase interaction versus PROTACS use of heterobifunctional molecules that recruit substrate and E3-ligases and tether them together.

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result as CR8. A third screening strategy was also used, whereby chemical screening in hyponeddylated cells coupled to multi-omics approaches led to the identification of another MGD that promoted the interaction between CDK1-cyclin K and CRL4B ligase complexes[8]. CDK12-cyclin 12 axis regulates a host of cellular processes including proliferation and migration; importantly, it has been implicated in several cancers including breast and melanoma. Because of their critical role several small molecule inhibitors have been developed to inhibit their functions. Recently, the small molecule SR-4835 CDK12 inhibitor was shown to function as a MGD and was determined that its cytotoxicity depended on the CUL4-RBX1-DDB1 ubiquitin ligase complex[9]. It is quite interesting that distinct approaches all led to the identification of somewhat unique MGDs that all enhance interaction between CDK12-cyclin K and DDB1. In another recent study, a group sought to examine these and other molecules to gain a better understanding of the design principles for MGDs. They examined 91 MGDs in an array of structural, biophysical, and cellular studies and determined that these CDK12 MGDs appear to interact with Arg 928 on the interfacial region, and determined that the degree of CDK12 inhibition relative to cyclin K degradation is tunable which may be important therapeutically[10].

CK1α a Molecular Glue Degrader Target

The IMiD, lenalidomide, has been used as a critical treatment for multiple myeloma; however, its mechanism of action remained undetermined for many years. In 2014, two groups determined that lenalidomide enhanced the interaction between the zinc finger proteins 1 and 3 (IKZF1 and IKZF3) and cereblon[2,3]. Since then, lenalidomide has been shown to target several other proteins as well, some of which are now being examined as therapeutic targets[(reviewed in 1)]. One, in particular, is casein kinase 1α (CK1α), because it was shown to be important for lenalidomides effect in myelodysplastic syndrome (MDS) patients with deletion of chromosome 5q (del(5q)). This effect is noticeable because these types of MDS patients are haploinsufficient for CK1α. Importantly for CK1α to be a viable target, improved MGDs are needed as lenalidomide only had a modest effect on CK1α, thus it had minimal effect in AML models where CK1α levels are high and is thought to be important for disease progression. Nishiguchi et al. utilized a library of cereblon ligands and identified SJ7095 which is a potent degrader of Ck1α, IKZF1 and IKZF3[11]. They then utilized SAR to evolve the drug which resulted in SJ3149 a drug that co-crystalized with CRBN-DDB1 and in tests again over a hundred cancer cell lines they observed broad antiproliferative activity[11]. Another group also developed a specific MGD that targets CK1α and IKZF2, which they called DEG-35 (and its more soluble version DEG-77)[12]. Interestingly, it was shown to block cell growth and induce myeloid differentiation in AML lines, and also delayed leukemia progression in AML mouse models[12]. Several pharmaceutical companies are developing MGDs to specifically target IKZF1, IKZF3, and CK1α in hematologic cancers.

Future Directions

Targeted protein degradation as a drug modality is an emerging, yet highly promising field. It's been a decade since IMiD protein targets were first identified, and since then targets, MGD small molecules, and novel screening approaches have grown tremendously as discussed above. These efforts have translated into at least 12 MGD drugs in clinical trials by an array of pharmaceutical companies including Monte Rosa Therapeutic, BMS, C4 Therapeutics, and others for the treatment of hematologic and solid tumors. Multiple other protein targets are now under investigation. For example, GSPT1 and GSPT2, which may be implicated in acute lymphoblastic leukemia, has been targeted with an MGD, SJ6986, and the results have been promising[13]. Additionally, alternative molecular glue-like molecules and mechanisms are under intense investigation such as, this pre-print study where the group defined a trans-labeling covalent MG mechanism[14], or another study where dual-nanobody cannabidiol sensors were used to help define MGs[15]. The future of MGD research is very bright, and Cytoskeleton inc. is proud to provide important ubiquitination detection tools to assist with this research.

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Signal Seeker™ Ubiquitination Tools

Product	Amount	Cat. #
Ubiquitin Antibody Mouse Monoclonal (P4D1)	2 x 100 ul 1 x 25 ul 4 x 500 ul	AUB01 AUB01-S AUB01-XL
Ubiquitin-HRP Antibody Mouse Monoclonal (P4D1)	1 x 100 ul 1 x 25 ul	AUB01-HRP AUB01-HRP-S
Ubiquitination Affinity Beads	40 reactions	UBA01-beads
Ubiquitination Control Beads	10 reactions	CUB02-beads

Signal Seeker™ PTM Detection Kits

Product	Assays	Cat. #
Signal-Seeker™ Phosphotyrosine Detection Kit	30	BK160
Signal-Seeker™ Phosphotyrosine Detection Trial Kit	10	BK160-S
Signal-Seeker™ Ubiquitination Detection Kit	30	BK161
Signal-Seeker™ Ubiquitination Detection Trial Kit	10	BK161-S
Signal-Seeker™ SUMOylation 2/3 Detection Kit	30	BK162
Signal-Seeker™ SUMOylation 2/3 Detection Trial Kit	10	BK162-S
Signal-Seeker™ Acetyl-Lysine Detection Kit	30	BK163
Signal-Seeker™ Acetyl-Lysine Detection Trial Kit	10	BK163-S
Signal-Seeker™ SUMOylation 1 Detection Kit	30	BK165
Signal-Seeker™ SUMOylation 1 Detection Trial Kit	10	BK165-S

Signal Seeker™ PTM Affinity Beads

Product	Amount	Cat. #
Phosphotyrosine Affinity Beads	4 x 330 ul	APY03-beads
SUMO 2/3 Affinity Beads	2 x 400 ul	ASM24-beads
Acetyl-Lysine Control Beads	10 reactions	CIG02-beads
SUMO1 IP Control Beads	12 reactions	CIG03-beads