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COMMENTARY

Innovative discovery and mechanistic validation of HyT-PD ligands for selective CDK9-targeted protein degradation

KEY WORDS

Targeted protein degrader;
HyT-PDs;
ATG101;
CDK9;
Autophagy−lysosome pathway

Targeted protein degrader has emerged as a transformative therapeutic technology, offering a novel modality to address previously intractable drug targets and enabling innovative approaches to disease treatment[1]. Unlike conventional small-molecule drugs that function through occupancy-driven mechanisms, targeted protein degradation (TPD) employs an event-driven pharmacological strategy by harnessing the ubiquitin−proteasome system (UPS) and lysosomal degradation pathways[2]. This paradigm offers several intrinsic advantages, including the potential to overcome drug resistance, enhance pharmacological potency, and target traditionally undruggable proteins[3]. Several classes of degraders have been developed, including proteolysis-targeting chimeras (PROTACs), molecular glues, lysosome-targeting chimeras (LYTACs), antibody-based PROTACs (AbTACs), and hydrophobic tagging protein degraders (HyT-PDs)[4]. Among these, HyT-PDs are composed of a ligand for the protein of interest (POI), a linker, and a hydrophobic tag. These molecules are characterized by low molecular weight, favorable pharmacokinetic (PK) properties, and high drug-likeness[5]. However, the design principles and mechanistic underpinnings of HyT-PDs remain poorly understood, as they may engage multiple cellular degradation pathways, including UPS, autophagy, or alternative systems[5].

Recent efforts have focused on developing small-molecule inhibitors and degraders targeting cyclin-dependent kinase 9 (CDK9), a promising target in cancer and other diseases[6,7]. Nonetheless, challenges such as limited selectivity, toxicity, and insufficient mechanistic insight persist. A recent study by Zhong et al.[8] addressed these challenges by identifying a novel HyT-PD ligand, **AZ-9**, which selectively degrades CDK9 *via* the autophagy−lysosome pathway, providing robust mechanistic validation both *in vitro* and *in vivo*.

The authors initially identified **AZ-9** based on its ability to induce CDK9 degradation and inhibit cell proliferation. **AZ-9** demonstrated remarkable degradation efficiency, selectivity, and broad applicability, confirming its potential as a CDK9 degrader. Phenotypic assays further supported its activity by showing modulation of canonical CDK9 downstream pathways.

Mechanistic investigations revealed that **AZ-9** induces the degradation of CDK9 and its binding partner Cyclin T1 through the autophagy−lysosome pathway. Crucially, ATG101 was identified as a mediator recruited by **AZ-9** to initiate this process. The activation of autophagy was validated by the detection of LC3, a key biomarker of autophagosome formation[9], and direct visualization of autophagosome−lysosome fusion upon **AZ-9** treatment. These findings indicate that **AZ-9** facilitates ATG101 recruitment, thereby activating autophagy through LC3 engagement, promoting autophagosome formation, and ultimately leading to lysosomal degradation of CDK9 and Cyclin T1. To further support its translational potential, the authors evaluated the *in vivo* efficacy, selectivity, and safety profile of **AZ-9**. This study represents the first demonstration of a non-proteasomal degradation mechanism using HyT-PD technology to target CDK9, marking a significant advance in the field.

Despite these promising results, several limitations warrant further investigation. While the primary degradation pathway has been elucidated, the precise molecular interactions between **AZ-9** and **ATG101** remain unclear. Structural studies are needed

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to elucidate how **AZ-9** facilitates this interaction and whether this mechanism can be generalized to other proteins. Additionally, although **AZ-9** shows strong *in vivo* efficacy, further optimization may be required to enhance its clinical translatability.

In summary, Zhong et al. present compelling evidence for a hydrophobic tag-based degrader that selectively targets CDK9 *via* the autophagy−lysosome pathway. This work provides the first experimental validation of non-proteasomal degradation using HyT-PD technology and highlights **AZ-9** as a promising candidate for therapeutic development in CDK9-associated diseases.

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