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HIGHLIGHT

TRIM21-based degradation strategy for multimeric proteins



KEY WORDS

Targeted protein degradation;
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Targeted protein degradation (TPD) revolutionizes drug discovery by taking advantages of event-driven mechanism of action (MOA). Among current proximity-inducing platforms for TPD, molecular glue degraders and proteolysis-targeting chimeras (PROTACs) are representative strategies that facilitate interactions between an E3 ligase and a specific protein target. This leads to ubiquitination and subsequent degradation of the target by the ubiquitin–proteasome system (UPS). Despite offering unprecedented opportunities for more effective and targeted therapies, current UPS-hijacking TPD still faces key challenges. For instance, although over 600 E3 ligases have been identified in human cells, only a handful, such as cereblon (CRBN) and von Hippel-Lindau (VHL), are accessible for developing degraders¹. In addition, there are ongoing questions about how to further expand the chemically tractable target space beyond monomeric proteins with cytosolic domains.

In December 2024, Han and Huang's group published a novel study in *Cell*, introducing TRIM21 E3 ligase-based TPD as a promising approach for selectively degrading multimeric proteins, which has historically been a challenging issue². In this study, the authors identified (S)-ACE-OH, a metabolite of acepromazine (ACE), as a molecular glue that simultaneously recruits TRIM21 and the nucleoporin NUP98 (Fig. 1A). Then ACE was functionalized into TRIM21-based PROTAC degraders, termed TrimTACs, for the selective elimination of aberrant proteins in multimeric assemblies (Fig. 1B). This TrimTAC strategy offers exciting new

avenues for therapeutic intervention in multimer-related disorders, such as autoimmunity, neurodegeneration, and cancer.

Specifically, through an *in vitro* competitive cell growth assay, the authors identified three phenothiazine derivatives—acepromazine, acetophenazine, and piperacetazine—that selectively impaired the fitness of wild-type (WT) A549 lung adenocarcinoma cells compared to *IFNGR1*-deficient A549 cells in the presence of interferon gamma (IFN γ). Among them, ACE, a veterinary antipsychotic drug, was further confirmed to exhibit IFN γ -enhanced anticancer activity against A549 cells. This augmented cytotoxicity was also observed in three additional cancer cell lines: HuH-7, HeLa, and SiHa. Conversely, ACE did not exhibit such effect in the cancer cell lines DLD-1, ME-180, and Mino, suggesting a potential specificity in its mode of action. Further investigation revealed that the anticancer selectivity is due to the efficacy of the reductive metabolite of ACE, specifically (S)-ACE-OH. ACE-sensitive cells (e.g., A549 and HeLa) express high levels of aldo-keto reductases, which convert ACE to its active metabolite. However, ACE-insensitive cells (e.g., DLD-1 and HCT-116) lack these reductases.

The authors then elucidated that interferons enhance ACE's anticancer activity by inducing the expression of TRIM21, an E3 ubiquitin ligase critical to the host antiviral response. They hypothesized that the Fc-binding pocket within the PRYSPRY domain of TRIM21, particularly the D355 amino acid residue, plays a pivotal role in mediating the enhanced anticancer activity, potentially through specific protein–protein interactions (PPIs) involving ACE. To identify proteins degraded by TRIM21-facilitated mechanism, the authors performed proteomics analyses of TRIM21^{D355A}-overexpressed A549 cells treated with ACE at 10 μ mol/L for 8 h. Several nucleoporins, including NUP35, NUP155, SMPD4 and GLE1, exhibited significant lowering in abundance. Western blotting assay confirmed that (S)-ACE-OH, but not (R)-ACE-OH, significantly induced the degradation of these nucleoporins in IFN γ -pretreated A549 cells at a concentration of 20 μ mol/L.

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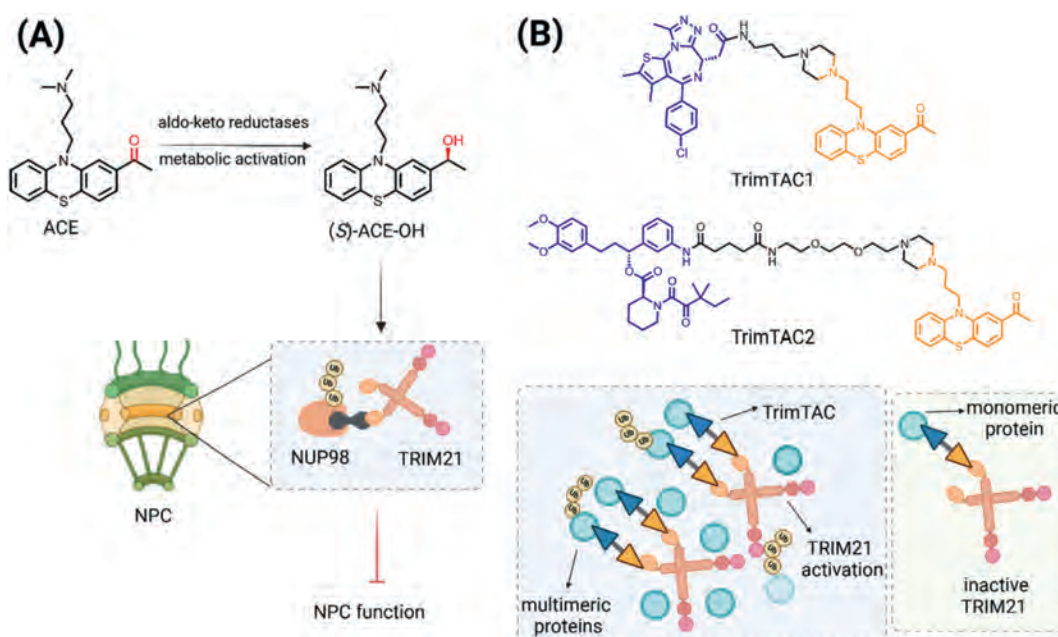


Figure 1 Mechanism of TRIM21-based degradation strategy. (A) TRIM21-based molecular glue (S)-ACE-OH degrades nucleoporins and damages the function of NPCs; (B) TRIM21-based PROTACs selectively degrade multimeric proteins without affecting monomeric proteins. The figure was created in BioRender. <https://BioRender.com/v45v052>.

In humans, nucleoporins are stationary components of nuclear pore complexes (NPCs), which facilitate nucleocytoplasmic exchange³. ACE treatment induced the degradation of nucleoporins and thereby damaged the internal structure of NPCs, leading to functional impairment of the nuclear pores. The authors then investigated whether and how the TRIM21-induced depletion of NPCs contributes to ACE's antitumor activity. Through a series of experiments, they demonstrated that mutations in nucleoporin NUP98, specifically at the site targeted by sgRNA against K752, confer resistance to ACE in A549-Cas9 cells. NUP98 is an important component of the NPC that undergoes post-translational autophosphorylation *via* its autophosphorylation domain (NUP98^{APD})⁴. The GST pull-down assay revealed that (S)-ACE-OH enhanced the interaction between TRIM21 and NUP98^{APD}. The CEBIT (condensate-aided enrichment of biomolecular interactions in test tubes) assay also supported NUP98^{APD} as the direct molecule glue target of TRIM21. To elucidate the differential biological activity between (S)-ACE-OH and (R)-ACE-OH, the authors performed isothermal titration calorimetry (ITC) analysis, which revealed that NUP98^{APD} exhibited strong binding affinity for the preformed TRIM21^{D355A}:(S)-ACE-OH complex ($K_d = 0.302 \mu\text{mol/L}$). In contrast, the binding of NUP98^{APD} to the TRIM21^{D355A}:(R)-ACE-OH complex was significantly weaker, exhibiting a 17-fold reduction in affinity. To further investigate these interactions, the authors determined the co-crystal structures of TRIM21^{D355A} PRYSPRY in complex with ACE, (S)-ACE-OH, and (R)-ACE-OH, respectively. Structural analysis showed that in both the ACE and (R)-ACE-OH complexes, the amine group extends outside the binding pocket. However, in the (S)-ACE-OH complex, the amine group engages in polar interactions with the side chains of E389 and Q395. These structural findings may provide a molecular basis for understanding why (S)-ACE-OH functions as the active metabolite, whereas (R)-ACE-OH remains inactive.

The co-crystal structures of TRIM21^{D355A} PRYSPRY bound to ACE revealed flexibility in ACE's aliphatic chain and extension of its amine group beyond the binding pocket, offering a feasible linkage site for PROTAC design. As a proof-of-concept study, the authors designed TrimTACs, PROTACs harnessing TRIM21, by chemically conjugating ACE with ligands specific to disease-associated targets bromodomain-containing protein 4 (BRD4) and FKBP12-binding protein 12 (FKBP12), respectively (Fig. 1B). These TrimTACs lost the ability to function as molecular glue degraders targeting nucleoporins. It is worth highlighting that TrimTACs could drive selective degradation of target proteins present in multimeric assemblies. Specifically, BRD4 targeting TrimTAC1 failed to degrade soluble mEGFP-BRD4^{BD2} in A549 cells but selectively degraded NUP98^{FG}-mEGFP-BRD4^{BD2} nuclear condensates. Interestingly, TrimTAC1 also selectively degraded BRD4^{BD2}-mEGFP-cGAS condensates upon DNA transfection, without effect on their levels in the absence of transfection, suggesting potential therapeutic applications in diseases caused by aberrant assemblies. Similarly, FKBP12 targeting TrimTAC2 effectively depleted NUP98^{FG}-mEGFP-FKBP12 condensates without affecting soluble mEGFP-FKBP12, further demonstrating the generalizability of the TrimTAC strategy.

In summary, this study introduces an innovative expandable TPD strategy that leverages TRIM21 to selectively degrade multimeric proteins within diverse biomolecular condensates, while sparing monomeric proteins in the dilute phase. Notably, TrimTAC expands the toolkit of accessible E3 ligases and broadens the target scope of TPD. However, since the TRIM21 ligand ACE only exhibits moderate binding affinity ($K_d = 5.66 \mu\text{mol/L}$) as measured by ITC analysis, there is considerable potential to further enhance the efficacy of this TRIM21-based TPD approach. Very recently, a study has demonstrated that two previously clinically-evaluated cytotoxic agents, PRLX-93936 and BMS-214662, also function as cytotoxic

molecular glues that specifically recruit TRIM21 to induce nucleoporin degradation⁵. Interestingly, PRLX-93936 and BMS-214662 exhibit significantly superior anticancer activity against OCI-AML-3 acute myeloid leukemia cells as compared to ACE-OH. This remarkable potency, combined with their established clinical safety profiles, positions PRLX-93936 and BMS-214662 as promising chemical scaffolds for the rational design of innovative aggregate-targeting PROTACs.

Author contributions

Wei Wang: Writing - original draft, review & editing. Chunquan Sheng: Writing - review & editing, supervision, conceptualization.

Conflicts of interest

The authors declare no conflicts of interest.

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