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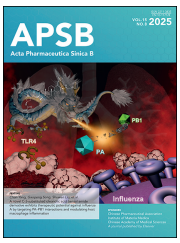

EDITORIAL

HomoPROTAC^{Keap1}: A new strategy of chemical knockdown of Keap1 for allergic rhinitis

KEY WORDS

Keap1;
HomoPROTAC;
Allergic rhinitis;
Nrf2

Allergic rhinitis (AR) has been characterized as being caused by an immunoglobulin E (IgE)-mediated immune inflammation to inhaled allergens, which causes an underestimated and substantial economic burden and disability worldwide. For clinical management, continuous allergen avoidance should be the first choice for AR patients. Many pharmacotherapy options are available for AR to encompass symptomatic management, such as hormone therapy, targeted drug therapy, and immunotherapy. However, hormones such as dexamethasone can only control symptoms in the short term, and prolonged use may lead to systemic side effects; the effects of many targeted drugs are uncertain, and the cost is high; immunotherapy is time-consuming and costly; and these methods involve the use of systemic drugs, with side effects that are not conclusive. Scientists globally have embarked on a relentless effort to discover novel therapeutic interventions.

A recent proof-of-concept study led by Professor Chunlin Zhuang from the Second Military Medical University reported an innovative targeted protein degradation strategy for treating AR: homobivalent proteolysis-targeting chimeras degrading Keap1 (homoPROTAC^{KEAP1})[1]. In the study, it was confirmed that KEAP1-related oxidative stress and inflammation are dysregulated in the peripheral blood and nasal swabs of AR patients. Targeting oxidative stress signaling and related enzymes such as nuclear factor erythroid 2-related factor 2 (Nrf2) may be effective for AR intervention. Nrf2, a key regulator in the oxidative stress pathway, is inhibited by Kelch-like ECH-associated protein 1 (Keap1) to maintain cell homeostasis. Meanwhile, Keap1 functions as a homodimerization adaptor protein within the Cullin−RING E3 ubiquitin ligase system, facilitating the ubiquitination of Nrf2. In the crystallographic study, researchers further identified a unique dimerization pattern of the Keap1 protein with a "face-to-face" configuration, confirming the existence of the Keap1 dimer. The research leveraged this property to design bifunctional molecules that bind two molecules of Keap1 and aim to achieve chemical knockdown of Keap1 by harnessing the ubiquitin−proteasome system, potentially representing a groundbreaking strategy for the treatment of AR.

The study demonstrated successful crystallography-driven design of compounds as homodimer inhibitors of the Keap1−Nrf2 interaction. One notable example was the design of compound **8**, a dimer synthesized through a single-sided linkage at the amino group present on the benzosulfamide moiety through a polyethylene glycol (PEG) linker. Compound **8** excelled with the best K_{D2} value of 41 nmol/L among the same type of divalent compounds, outperforming the monovalent NXPZ-2, which had a K_{D2} of 53 nmol/L. Microscale thermophoresis (MST) was used as the second biophysical method, and the same results were obtained. The MST traces and fitted K_d values were 45 nmol/L for NXPZ-2 and 29 nmol/L for compound **8**. It is worth noting that they determined a ternary crystal structure of the Keap1 dimer in complex with a single molecule of compound **8** at a resolution of 3 Å for the first time. The elucidation of the protein-ligand interaction mode has provided accurate crystal structure information and is regarded as a gold standard. The ternary dimer crystal structure was not only similar to the arrangement pattern of the dimer crystal, but also formed an interaction similar to Keap1−NXPZ-2 in the binding moiety between Keap1 and compound **8**. The hydrogen-bonding interactions between residues of Keap1 and the linker moiety of compound **8** were clearly illustrated, improving the interactions between Keap1 and compound **8**. The presentation of the crystals provided a very clear

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explanation of the conclusion reached in the size exclusion chromatography (SEC) experiment, which was used to validate the ability of the bivalent compound **8** to induce the formation of the ternary complex $(Keap1)_2$:(compound **8**)$_1$.

More importantly, the study also incorporated the property of Keap1 as an E3 ubiquitin ligase. Compound **8** possessed the best self-degradation efficiency as a homoPROTAC in MDA-MB-231 cells. The concentrations of 0.03 μmol/L and 0.1 μmol/L ($D_{max} > 90\%$) at 24 h showed the best efficacy. The homo-PROTAC^{KEAP1} exerted extensive self-destruction of Keap1 in a panel of cell lines. The DC_{50} values were determined as 3.17 μmol/L in Beas-2B cells, 1.64 μmol/L in HNEpC cells, 1.11 μmol/L in LLC cells, and 2.98 μmol/L in RAW264.7 cells. The researchers further revealed that the extensive self-destruction of Keap1 was mediated by the ubiquitin−proteasomal system. After treating different cells with either the ubiquitin inhibitor (MLN4924) or the proteasome inhibitor (MG132), the Keap1 degradation caused by compound **8** was reversed. As a bivalent compound for degradation, like a strategy for chemical knockdown, the efficiency of compound **8** was very similar to the biological knockdown of Keap1 (shKeap1, a Keap1-KD cell line through lentivirus infection). The degradation, leading to Nrf2 nuclear translocation, was also confirmed by shKeap1. The downstream HO-1 and NQO1 were significantly increased, and the binding of Nrf2 to the downstream antioxidant response element (ARE) was also increased after compound treatment. The regulation of the Keap1−Nrf2−ARE pathway caused by the degradation of Keap1 enabled the compound to exhibit anti-inflammatory activity in vitro and mitigate allergic nasal inflammation *in vivo* (OVA-induced AR mouse model).

This research is of great significance. Currently, there are few compound molecules specifically targeting allergic rhinitis. The proposal of this concept provides a novel homoPROTAC-mediated strategy for the study of clinical diseases. Through the first characterization of the $(Keap1)_2$:$(homoPROTAC)_1$ ternary complex, the potential binding mechanism is confirmed, which also provides valuable data support for further structural modification and research. Furthermore, in patients with clinical AR, dysregulation of Keap1-related oxidative stress and inflammation is observed, which confirms the biological importance of Keap1 and its role in AR. Intriguingly, this new molecule has been successfully applied to the OVA-mediated AR mouse model, and mRNA-seq analysis shows that it has good efficacy in regulating oxidative stress and alleviating AR symptoms. At the mechanistic level, it reveals the complex interaction between oxidative stress and inflammatory response in human allergic rhinitis and provides new ideas for the treatment of this common disease. Moreover, compared with biological knockout, this breakthrough design of small chemical degradation molecules has more advantages. Due to its convenient administration, it holds greater potential for clinical treatment development.

Despite all these advantages, there are several challenges for researchers. First, the large molecular weight of targeted degradation chimeras may lead to poor physicochemical properties such as drug formation. In this study, intranasal administration may be a feasible treatment approach, and a variety of administration methods can highlight its development potential. Second, collecting lesions of allergic rhinitis, such as nasal mucosa, can better reflect the biological information of inflammatory genes in the progression of AR. Third, the binding and degradation activities of the ternary complex were verified at the molecular level, but the precise recruitment and degradation of Keap1 in cells were not fully demonstrated.

Collectively, this work by Zhuang's group designed and synthesized a homoPROTAC molecule based on the Keap1 E3 ubiquitin ligase to induce its own degradation and elucidated its application as a new tool for Keap1 chemical knockout. It also expanded the E3 ubiquitin ligase toolbox for PROTAC research and provided a new strategy for the treatment of inflammatory diseases.

Reference

1. Yan JY, Wang TY, Yu RZ, Xu LJ, Shao HM, Li TF, et al. Chemical knockdown of Keap1 and homoPROTAC-ing allergic rhinitis. *Acta Pharm Sin B* 2025;**15**:4137−55.

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