Harnessing Antibody-Mimetic Selectivity for Activation-State-Specific Targeted Degradation of Endogenous K-Ras

The Ras family of GTPases are important but poorly druggable therapeutic targets that are frequently mutated in cancer. In this issue of ACS Central Science, Lim et al. present the development of a panel of biodegraders that trigger rapid degradation of endogenous K-Ras proteins with conformational specificity. The Ras GTPases (K-Ras, H-Ras, and N-Ras) cycle between the active GTP-bound state, which interacts with downstream effectors to promote cell growth, differentiation, and survival, and the inactive GDP-bound state. Activating mutations in Ras proteins are prevalent oncogenic drivers in a number of tumor types including pancreatic ductal adenocarcinoma (K-Ras), head and neck squamous cell carcinoma (H-Ras), and cutaneous melanoma (N-Ras). Small-molecule Ras inhibitor development is challenging due to the picomolar affinity these enzymes have for guanine nucleotides, which are present at high concentrations in the cell (0.1–1 mM) and compete with inhibitors for active site binding. Covalent inhibitors of the K-RasG12C mutant have shown promise in nonsmall cell lung cancer patient trials and provide clinical validation of K-Ras inhibition. However, other Ras mutants that lack a reactive cysteine proximal to the active site remain undrugged despite intensive efforts in the medicinal chemistry community.

Targeted protein degradation (TPD) has emerged as a new paradigm in drug discovery which circumvents the need for high target occupancy. Here, a heterobifunctional small molecule (degrader) recruits a protein of interest (POI) to an E3-ubiquitin ligase. The induced proximity results in the ubiquitination of the POI, and its subsequent proteasomal degradation. Limitations of TPD include the requirement for small-molecule binders of the target protein and E3 ligase of interest, and the unknown compatibility of the target:E3 ligase pair. Lim et al. describe a workflow addressing these challenges, culminating in the development of a panel of K-Ras-targeting bifunctional proteins they term “biodegraders”, which combine the exquisite potency and selectivity of biologicals with a targeted protein degradation strategy. Biodegraders are engineered proteins composed of a protein-based POI recognition domain such as a nanobody, and an E3-ligase substrate adaptor protein, which promotes ubiquitination of the recruited POI. First, the authors systematically examined the degradation activity of a panel of 10 E3 ligase substrate adaptor proteins fused with a GFP-nanobody against GFP-tagged K-Ras, to experimentally determine the optimal E3-ligase for inducing degradation. Speckle-type POZ protein (SPOP) was selected, and mutational studies confirmed the dependence of K-Ras-GFP degradation on both E3-ligase activity and GFP-nanobody affinity. Having demonstrated that SPOP
could degrade GFP-tagged K-Ras, the authors then set about re-engineering their system for the degradation of endogenous Ras proteins. A panel of six high-affinity Ras binding proteins, each with a different conformational, isoform, or mutant specificity, were identified from the literature and validated using isothermal titration calorimetry. Remarkably, for five of these binders, expression as fusion chimeras with SPOP afforded active biodegraders, highlighting the robustness of the approach (Figure 2). Induction of expression following transfection of plasmid DNA or direct transfection of mRNA resulted in degradation within 4 h, approaching the kinetics of small-molecule systems.

Previous efforts at developing biodegraders and PRO-TACs for K-Ras by recruiting other ligases have resulted in weaker degradation.1 However, this study identified numerous K-Ras compatible E3 ligases including VHL, which was later validated by the development of both chemical and biological degraders.7,8 This work provides an important proof-of-concept that chemical-genetic screening approaches can identify optimal target:E3 ligase pairs for challenging targets, which can be rapidly translated into degraders of endogenous proteins.

Lim and colleagues then used a panel of NanoLuc-Ras variant expressing cell lines to elegantly demonstrate that the biodegraders target Ras isoforms, mutants, and nucleotide-bound states according to the reported binding selectivity of the conjugated binder protein. A GDP-loaded K-Ras selective degrader was used to investigate the relative cellular prevalence of inactive K-Ras across a panel of oncogenic mutants, which largely recapitulated previously reported biochemical rank-ordering of their GTP-hydrolysis rates.9

Questions that remain to be explored are the effects of K-Ras biodegrader expression on the proteome to address the global selectivity of the biodegraders, and if they alter the levels of the endogenous substrates of the co-opted E3-ligases.

Small-molecule ligand discovery against a new target is a resource-intensive process, and up to 80% of the proteome is still considered undruggable.10 However, advances in
protein engineering technology have enabled the development of potent and selective protein binders against a vast array of proteins. By providing a blueprint for combining these two technologies to investigate uniquely challenging targets with isoform and conformational specificity, Lim et al. expand the potential target space of biodegrader-mediated TPD. Looking forward, applications of this approach may find utility for studying other high-value unliganded targets, for illuminating the functions of poorly understood proteins that lack chemical tools, and for prioritizing E3-ligases for ligand development efforts.

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**Notes**

The author declares the following competing financial interest(s): F.M.F. is a consultant to RA Capital.

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