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Engineering ubiquitin to modulate the ubiquitin proteosome system

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Ubiquitination determines the longevity of proteins in cells and plays major roles in signaling.[1,2] The complexity of the ubiquitination proteasome system (UPS) is evident in the number of enzymes that are involved, including hundreds of ligases and roughly a hundred deubiquitinases (DUBs) that add or remove ubiquitin chains, respectively. Furthermore, ubiquitin modifications on proteins are recognized by ubiquitin binding domains to direct a wide array of cellular processes, such as protein degradation, endocytosis, vesicular trafficking, cell cycle control, stress response, DNA repair, transcription and gene silencing.[3]

Members of the ubiquitin-specific protease (USP) family,[4] the largest DUB structural class, are involved in cancer and other diseases and many have been proposed as potential therapeutic targets.[5] However, much of the work implicating USPs and other DUBs in disease has relied on RNA interference approaches that act at the mRNA level. In contrast, virtually all drugs work at the protein level by targeting enzyme active sites or protein-protein interactions. Unfortunately, only a few weak inhibitors of DUBs have been identified to date,[6] and, consequently, their therapeutic potential has remained untapped. However, recent advances may change this situation dramatically, at least at the research level, as it has now been shown that ubiquitin itself can be used as a scaffold for generating inhibitors of USPs and other UPS enzymes.

Ubiquitin interacts weakly but specifically with thousands of proteins, and, in the case of USPs, structural analyses reveal that the enzymes recognize a common surface on the ubiquitin substrate but do so by means of binding sites that differ greatly across the class. Two recent reports show that engineered ubiquitin variants with sequence changes across this surface can act as potent inhibitors of specific USPs[7,8] (**Fig. 1**). Libraries encoding for billions of phage-displayed ubiquitin variants were selected for binding to USPs, and the selections yielded variants that bound tightly to a particular USP but did not recognize other closely related enzymes. The variants were found to be potent inhibitors of catalytic activity in vitro, and, as expected, structural analyses revealed that they acted as competitive inhibitors that directly block the substrate-binding site of the USP. Most importantly, ubiquitin variants that targeted three distinct USPs were shown to function as inhibitors in cells, where they elicited dramatic phenotypic responses.

In our own study,[7] we went further and showed that the ubiquitin-based inhibitor strategy can be extended to other DUB families, suggesting that the entire set of DUBs may be targeted in this fashion. In the case of the ovarian tumor protease family member OTUB1, structural analyses revealed that the ubiquitin variant bound to a distal exosite, and, consequently, not only inhibited the proteolytic activity of the enzyme, but also attenuated binding to an E2 ubiquitin-conjugating enzyme. Moreover, we extended the approach to E2 enzymes, E3 ubiquitin ligases and non-catalytic ubiquitin-binding domains. Unexpectedly, we found that a variant that targeted the E3 HECT ligase NEDD4 acted as a potent activator in cells, demonstrating that the approach can yield both inhibitors and activators.

In summary, ubiquitin can be used as a scaffold to rapidly develop potent and specific modulators of virtually any enzyme in the UPS. Ubiquitin variants can be thought of as a cross between small molecules and antibodies, and as tool compounds, they hold advantages over both. Like small molecules, the variants act at the protein level to modulate catalytic function. Consequently, they can be used to assess directly the effects of active site inhibition for target validation, and insights from structural studies may be applicable to the design of mechanism-based therapeutic inhibitors. Like antibodies, ubiquitin variants can be used in cell biology experiments for elucidating the biological functions of enzymes in the UPS. However, the recombinant variants are more versatile than antibodies, because they are well adapted for function inside cells. Thus, ubiquitin variants should enable rapid target validation and biological discovery within the UPS.

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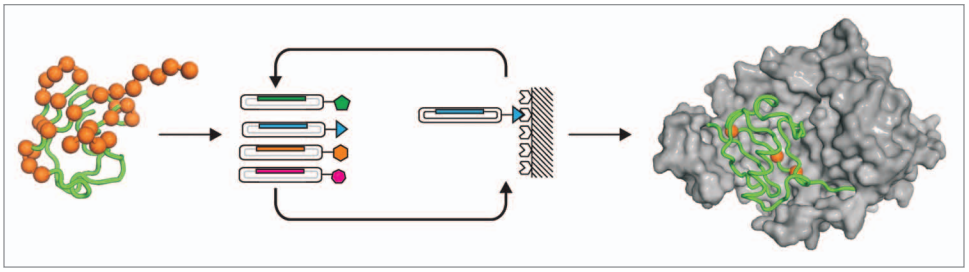

Figure 1. Development of ubiquitin variants as USP inhibitors. The USP-binding surface of ubiquitin was combinatorially mutated (left) to generate a phage-displayed library that was used to select variants (center) that inhibit USP activity by targeting the substrate-binding site (right). The ubiquitin main chain is shown as a green tube and diversified positions in the library are shown as orange spheres. USP21 is shown as a gray surface in complex with an inhibitory ubiquitin variant (PDB entry 3MTN). Phage particles are shown as rectangles with the ubiquitin-variant-encoding DNA inside, and the colored symbols represent different ubiquitin variant proteins displayed on the phage surface.