Protacs: Chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation

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The intracellular levels of many proteins are regulated by ubiquitin-dependent proteolysis. One of the best-characterized enzymes that catalyzes the attachment of ubiquitin to proteins is a ubiquitin ligase complex, Skp1-Cullin-F box complex containing Hrt1 (SCF). We sought to artificially target a protein to the SCF complex for ubiquitination and degradation. To this end, we tested methionine aminopeptidase-2 (MetAP-2), which covalently binds the angiogenesis inhibitor ovalicin. A chimeric compound, protein-targeting chimeric molecule 1 (Protac-1), was synthesized to recruit MetAP-2 to SCF. One domain of Protac-1 contains the IκBα phosphopeptide that is recognized by the F-box protein β-TRCP, whereas the other domain is composed of ovalicin. We show that MetAP-2 can be tethered to SCF$^{\beta\text{-TRCP}}$, ubiquitinated, and degraded in a Protac-1-dependent manner. In the future, this approach may be useful for conditional inactivation of proteins, and for targeting disease-causing proteins for destruction.

Degradation of cellular proteins is required for normal maintenance of cellular function, including proliferation, differentiation, and cell death. One of the major pathways to regulate proteins posttranslationally is ubiquitin-dependent proteolysis. Ubiquitination occurs through the activity of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin–protein ligases (E3), which act sequentially to catalyze the attachment of ubiquitin to lysine residues of substrate proteins (1). The E3s confer specificity to ubiquitination reactions by binding directly to substrate. Although the exact number of E3s cannot be determined with certainty from sequence data, there are probably >100 distinct F-box-containing E3s encoded within the human genome (2). One particular class of E3s, the heterotetrameric Skp1-Cullin-F box

to be the primary target of the potent angiogenesis inhibitors fumagillin and ovalicin (OVA; refs. 7 and 8). Both of these compounds inhibit MetAP-2 by covalently binding His-231 in the active site. The consequent reduction in MetAP-2 activity is thought to block endothelial cell proliferation by causing p53-dependent arrest in the G_1 phase of the cell cycle (9). Importantly, MetAP-2 is not known to be ubiquitinated or a substrate for any SCF complex.

To determine whether MetAP-2 could artificially be targeted to SCF$^{\beta\text{-TRCP}}$, we synthesized proteolysis-targeting chimeric molecule 1 (Protac-1) that contained both the IPP and OVA. We hypothesized that the phosphopeptide moiety would bind β-TRCP, and the OVA moiety would bind MetAP-2, thereby recruiting MetAP-2 to SCF$^{\beta\text{-TRCP}}$ for ubiquitination (Fig. 1*A*). We reasoned that this strategy might work because synthetic ligands that link distinct proteins have been shown to be capable of regulating signaling pathways *in vivo* (10). In this article, we report that Protac-1 indeed binds MetAP-2 to SCF$^{\beta\text{-TRCP}}$ and thereby promotes MetAP-2 ubiquitination and degradation. Demonstrating that Protac-1 mediates the ubiquitination and degradation of a foreign substrate by SCF provides a basis to begin testing Protacs *in vivo* in addition to other targets known to promote disease.

Materials and Methods

Synthesis of IκBα-OVA Protac. OVA (1.4 mmol) was dissolved in 10 ml of methanol at 0°C, and $NaBH_4$ (3.0 mmol) was added slowly. After 30 min of stirring, methanol was removed under reduced pressure, and the resulting crude product was purified by flash column chromatography to yield ovalicinol (1.15 mmol, 82%). Fmoc-Gly was coupled to the ovalicinol to give Fmoc-Gly-