27O-ISMS21 **In Vivo Protein Knockdown by Chimeric Small-compound SNIPERs**

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Selective degradation of pathogenic proteins with small molecules is a novel approach for drug development. We have developed a protein-knockdown system based on hybrid small-molecule SNIPERs (Specific and Nongenetic IAP-dependent Protein Erasers) that recruit inhibitor of apoptosis protein (IAP) ubiquitin ligases to specifically degrade targeted proteins. These compounds are chimeric molecules containing two different ligands connected by a linker; one is a ligand for an E3 ubiquitin ligase IAP and the other is for a target protein of interest, which are designed to cross-link these proteins to induce polyubiquitylation and proteasomal degradation of the target proteins in cells. Here, we extend our prior study to show a proof of concept of the SNIPER technology *in vivo*. By incorporating a high affinity IAP ligand (LCL161 derivative), we developed a novel SNIPER against estrogen receptor α (ERα), SNIPER(ER)-87, that has a potent protein knockdown activity. The SNIPER(ER) reduced ERα levels in tumor xenografts and suppressed the growth of ERα-positive breast tumors in mice. With this IAP ligand, potent SNIPERs against other pathogenic proteins, BCR-ABL, bromodomain-containing protein 4 (BRD4), and phosphodiesterase-4 (PDE4) could also be developed.

By derivatizing the IAP ligand module, we further developed novel SNIPER(ER)s with superior protein knockdown activity. These SNIPER(ER)s show more sustained activity than SNIPER(ER)-87 to down-regulate ERα protein. They have higher binding affinities to IAPs, especially to cIAP1, than SNIPER(ER)-87, and effectively induce simultaneous reduction of ERα , cIAP1, and to a lesser extent XIAP, which results in growth inhibition and apoptosis induction in human breast cancer cells that require IAPs for cell survival. Mechanistic analysis reveals that the SNIPER(ER)s preferentially recruit XIAP rather than cIAP1 to degrade ERα as with SNIPER(ER)-87. These results indicate that forced ubiquitylation by SNIPERs is a useful method to achieve efficient protein knockdown with a potential therapeutic activities. This technology could also be applied to study the role of ubiquitylation in many cellular processes.