MODULAR DESIGN OF LIGAND-TOXIN CONJUGATES

Christopher D. Warner, Megan C. Larisch, and William W. Ja
Department of Metabolism and Aging, The Scripps Research Institute, Jupiter, FL, U.S.

Ligand-toxin conjugates—compounds that link potent cytotoxins to targeting ligands that impart cell-specificity—are useful for a variety of applications, from cancer therapeutics to animal chemosterilants. Most antibody-toxin (immunotoxin) conjugates are designed as fusion proteins, requiring laborious production strategies for screening numerous ligand-toxin pairs for the most promising therapeutic leads. Here, we use a leucine zipper, composed of acidic and basic "Velcro" peptide sequences, as a heterodimerization domain to fuse ligands with the *Pseudomonas* exotoxin fragment, PE38. PE38, a popular toxic warhead used in several cancer-targeting drugs, is produced in *E. coli* as a recombinant protein fused with the acidic Velcro sequence (av-PE38). This protein is immediately amenable to conjugation with any protein, peptide, or small molecule ligand that harbors the partnering basic Velcro (bv) peptide. To further accommodate "mix and match" studies with different ligands, we have synthesized the bv peptide with an alkyne-reactive azide to take advantage of click chemistry for rapidly ligating other molecules to the heterodimerization domain. Here, we demonstrate 1) the purification of av-PE38 fusion protein and its undiminished potency in inhibiting *in vitro* translation, 2) the chemical ligation of the peptoid, GU40C, to the bv peptide, and 3) the conjugation of av-PE38 to GU40C-bv. All research tools from this proof-of-principle study are being made available to the scientific community and we anticipate that the system will be used to quickly test hypotheses and screen promising targets for potential drug leads that may be useful for suppressing reproduction in dogs and cats.