MOLECULAR ENGINEERING

Knocking down Goliath

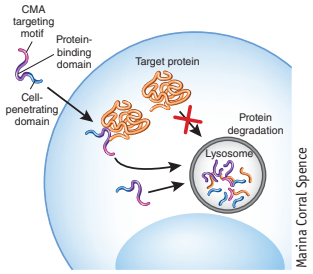
A small peptide–based method knocks down native proteins *in vitro* and *in vivo*.

Elucidating the role of a particular protein in physiological processes, as well as in the pathogenesis of disease, involves devising ways of knocking the protein down. But eliminating specific proteins in cells is not exactly easy, especially when one wants to target unmodified native proteins and to block their activity in a reversible, temporally controlled and specific manner. Methods that rely on DNA or mRNA knockdown, for example, are slow and generally target all isoforms of the protein. As in the biblical myth of the giant Goliath—who was defeated with a small stone thrown from a sling—Yu Tian Wang and his colleagues at the Vancouver Coastal Research Institute and University of British Columbia, now show how one can use small peptides to knock down proteins in cells and living animals.

The method takes advantage of the chaperone-mediated autophagy (CMA) mechanism that degrades specific proteins containing a certain pentapeptide in the lysosome. This pathway is particularly active in cells during stress and pathological conditions and has been used by researchers in the past to mediate the degradation of specific proteins engineered to contain the target motif. Wang and his team asked whether this system could be used more generally to degrade endogenous, unmodified proteins. Their strategy consisted of designing peptides that bind to a protein of interest and contain the CMA targeting motif such that degradation of the protein would be mediated through its interaction with the peptide.

They tested this idea on the protein DAPK1 (death-associated protein kinase 1), which is activated in the brain during stroke. Upon activation, DAPK1 binds the NMDA (*N*-methyl-D-aspartate)-type glutamate receptor complex, leading to cell death. Wang and colleagues wanted to test the cell-protective effects of knocking down activated DAPK1 in these conditions.

"The method relies heavily on a good design of the protein-binding peptide," says Wang. In this case, Wang and his team based the design on the known interaction between activated DAPK1 and the glutamate receptor, and they used the DAPK1 binding domain of the receptor as the protein-binding peptide. Although this strategy works well for proteins that have known interacting partners, phage-display or peptide array–based screening methods can also yield good candidate peptides, says Wang.



Degradation of proteins using small peptides.

To avoid the need for expressing the peptide exogenously, the team then grafted on the cell membrane–penetrating sequence TAT and purified or synthesized the resulting peptides. The authors administered the peptides—containing the TAT and CMA motifs and the protein-binding domain—to cell lines and primary neuronal cultures and were able to knock down more than half of the DAPK1 protein in just a few hours. This effect was specific to the active DAPK1 protein, reversible, dose dependent and mediated by the lysosome.

The team used the same basic principle to design peptides that knocked down two other native proteins in cells. They then tested the effect of knocking down activated DAPK1 by systemic administration of the peptide to rats that had suffered from an ischemic insult, and the team was able to observe neuroprotective effects of the peptide.

Looking to the future, Wang and his colleagues want to apply the approach to target pathogenic proteins such as mutant huntingtin. They are also thinking of ways to refine the system by enabling selective targeting of cells or making it drug inducible. The approach could also be useful for basic studies of protein function and may serve as inspiration for other uses of small peptides to control larger protein beasts.

Erika Pastrana

RESEARCH PAPERS

Fan, X. *et al.* Rapid and reversible knockdown of endogenous proteins by peptide-directed lysosomal degradation. *Nat. Neurosci.* doi:10.1038/nn.3637 (26 January 2014).