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Point of VIEW

Disrupting Proteins To Treat Cancer

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ABSTRACT Multiprotein complexes figure prominently in all cellular processes. Disrupting formation of these complexes can modulate key cellular pathways and offers new possibilities for therapeutic intervention. A new study illustrates an efficient approach for developing high-affinity mimics that inhibit protein−protein interactions.

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Recent genome-wideproteomic studies tell us that the majority of proteins in eukaryotic cells physically interact with at least one partner (*1*). Together with genetic approaches that probe functional relationships, these studies reveal an extensive network of protein interactions and identify mechanisms of cross-talk between cellular processes. This information opens up opportunities for developing molecules that disrupt key interactions involved in disease pathways. On page 161 of this issue, Cortajarena *et al.* use protein engineering to design a high-affinity inhibitor for the interaction between the 90 kDa heat shock protein (Hsp90) and Hsp organizing protein (HOP). By disrupting the HOP−Hsp90 interaction, the molecule developed by Cortajarena *et al.* reduces cellular levels of a growth factor receptor that figures predominantly in breast cancer, and kills cultured breast cancer cells.

Hsp90 is a molecular chaperone. Chaperones are a class of ATP-dependent enzymes that assist in the folding and maturation of client proteins. Many proteins depend upon this activity in order to function, and Hsp90 is essential for eukaryotic cell viability. Indeed, it is one of the most abundant proteins in human cells. Hsp90 operates at the end stages of folding. Newly synthesized proteins bind to Hsp70, another chaperone. By an ATP-driven mechanism that is mediated by co-chaperones, Hsp70 prevents aggregation of the bound protein, resolves misfolded structure, and facilitates proper folding. The protein is then handed off to Hsp90. Hsp90 binding stabilizes the protein and maintains it in the proper conformation for function (*2*). This process is thought to involve an ATP-dependent "clamping" action, although the structural and biochemical details are not yet known.

Among the ~100 known clients of Hsp90 are proteins that promote cell growth and survival. Not surprisingly, cancer cells often have elevated levels of Hsp90. One client protein is human epidermal growth factor receptor-2 (HER2). HER2 is overexpressed in 25–30% of breast cancers, and increased levels of HER2 are associated with inferior prognosis (*3*). Both Hsp90 and HER2 have thus been a focus of drug design efforts. Molecules that inhibit Hsp90 ATPase activity are in clinical trials (*4*). A monoclonal antibody against HER2 (trastuzumab, also known as Herceptin) is in widespread use, and molecules that inhibit HER2 kinase activity are undergoing development (*3*). The protein created by Cortajarena *et al.* does not target enzyme function, so it can potentially be used in conjunction with ATPase and kinase inhibitors.

Hsp90 has no chaperone activity on its own. HOP provides the physical linkage between Hsp90 and Hsp70 that is necessary to coordinate their functions. HOP binds to both proteins *via* a common protein interaction module known as the tetratricopeptide repeat (TPR) domain. TPR motifs, named for their 34-amino acid repeating units, consist of two interacting, antiparallel α-helices (helix A and helix B). The functional domain is assembled from 3–16 TPR motifs packed in a parallel fashion (Figure 1, panel a). The

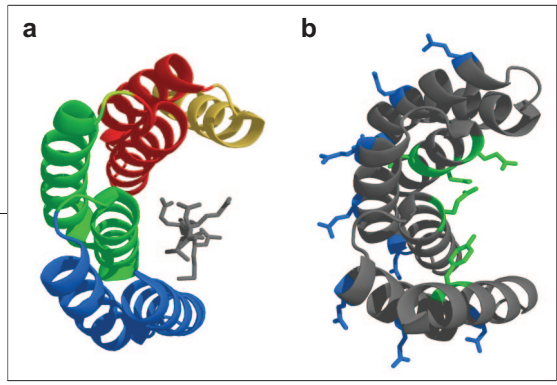

Figure 1. X-ray structures of TPR domains. a) Structure of the HOP TPR2A domain bound to the MEEVD peptide from Hsp90 (*8*). The three TPR motifs are colored blue, green, and red. The extra C-terminal helix shown in yellow caps the last TPR helix and increases solubility of the protein. b) Structure of the idealized TPR domain CTPR3 (*7*). Side chains in green indicate positions where Cortajarena *et al.* grafted binding residues from TPR2A to generate CTPR390. Side chains in blue denote solvent-exposed positions where the same researchers substituted positively charged amino acids to generate CTPR390+.

A helices form a concave surface to which the target peptide binds. The TPR-binding peptides from Hsp90 and Hsp70 are very similar. They are located at the extreme C-terminus of each protein, and they are composed primarily of the EEVD sequence. Nevertheless, the TPR domains of HOP can discriminate between the two ligands: TPR1 binds the TIEEVD peptide from Hsp70, while TPR2A recognizes the MEEVD peptide from Hsp90 (*5*).

The strategy taken by Cortajarena *et al.* was established >10 years ago, when Chen *et al.* showed that the isolated TPR domain from protein phosphatase 5 (PP5) acts as a dominant negative mutant (*6*). PP5 is an enzyme that binds to the same MEEVD sequence of Hsp90 and works with Hsp90 to process steroid receptor proteins. When the TPR domain was overexpressed in cells, it out-competed endogenous PP5 for binding to Hsp90 and thus inhibited proper folding of steroid receptors.

In order to generate inhibitors with greater affinity and specificity, Regan and coworkers had to make several key design improvements. They first created an extremely stable, idealized TPR motif to be used as a template (*7*). The idealized TPR sequence was generated from a statistical analysis of 1837 TPR motifs from 107 proteins. Structural and biophysical studies confirmed that the new sequence retained the TPR fold and that stability increased with addition of TPR subunits. The idealized three-TPR protein (CTPR3) exhibited a melting temperature of 83 °C, compared with a value of 47–50 °C for the similar-sized TPR domains from PP5 and HOP.

Cortajarena *et al.* then "tuned" CTPR3 for binding to Hsp90. They grafted onto it five Hsp90-binding amino acids from the TPR2A domain of HOP to create the CTPR390 protein. The binding residues (colored green in Figure 1, panel b) are all located in the A helices. CTPR390 bound the MEEVD peptide but with 40-fold less affinity than TPR2A. How then can affinity be improved?

To address that problem, Cortajarena *et al.* turned to the B helices. Residues in the B helices do not make direct contact with the substrate. They can, however, contribute to binding by forming long-range electrostatic interactions. CTPR390+ and CTPR390− were created by substituting basic and acidic residues, respectively, at solvent-exposed positions in the B helices. In this way, the back faces of each molecule became almost entirely positively and negatively charged (Figure 1, panel b). CTPR390+ bound the negatively charged MEEVD peptide 5-fold more tightly than TPR2A. Importantly, binding specificity not only was retained but was improved. CTPR390+ showed at least a 100-fold preference for the Hsp90 peptide over the identically charged Hsp70 peptide, compared with the 10-fold preference exhibited by TPR2A. Clearly, binding affinity and specificity are dictated by both direct and indirect interactions formed by side chains on concave as well as convex surfaces of the TPR domain.

The design of CTPR390+ is noteworthy because charges are used to tighten binding to the ligand without modifying the amino acids at the physical binding interface. The high density of positive charges on the back face pulls the peptide against the binding surface formed by the A helices. The high stability of CTPR3 was no doubt critical to the success of this approach, because the penalty paid for concentration of like charges is thermodynamic destabilization. The work of Cortajarena *et al.* may facilitate development of potent inhibitors by allowing researchers to improve affinity without extensive redesign of binding interfaces.

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