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Breaking Down Tumor Defenses

BCL-2 antiapoptotic proteins are considered ripe targets for anticancer drugs, yet only recently have small molecule inhibitors emerged. Beccatini and colleagues [1] find a BCL-XL inhibitor in the guise of a familiar natural product, gossypol. An analog, apogossypol, is a relatively selective BCL-XL antagonist.

Current understanding of the cytotoxic effects of antineoplastic agents involves the activation of endogenous cell suicide programs (also known as apoptosis) by one or more damage-response pathways. The regulation of apoptosis depends on the balance of pro- and antiapoptotic proteins within the cell. In particular, the BCL-2 protein family, encompassing both pro- and antiapoptotic members, governs this important cell decision. Basal expression of BCL-X_L, a prominent antiapoptotic family member, has a strong negative correlation with sensitivity to multiple classes of chemotherapeutics in the 60 cell lines of the National Cancer Institute anticancer drug screen [2].

The greatest hurdle for the development of BCL-2 inhibitors was thought to be the proven difficulty of inhibiting protein-protein associations with small molecules [3]. Structures of the BCL-2 family members determined so far (BCL-2/BCL-X_L, BCL-W, BAX, BID) contain solvent-exposed hydrophobic grooves that bind to synthetic peptides derived from the conserved BH3 domain in proapoptotic BCL-2 family proteins with submicromolar to nanomolar affinities [4]. This peptide-protein association serves as a template for proposed structural models of dimers made up of proapoptotic and antiapoptotic BCL-2 proteins (Figure 1), generally understood as a mechanism of sequestering proapoptotic proteins intracellularly [5]. The peptide binding surface in the hydrophobic cleft covers ~1100 $Å^2$, typical of protein-protein interfaces. Despite these reservations, both in silico screening of compound databases and peptide-based competitive binding assays have generated lead compounds capable of dissociating BH3 peptides from BCL-2 binding pockets [6, 7]. Notably, small molecule inhibitors of BCL-X_L pore-forming activity cannot displace BH3 peptides, suggesting that there may be more than one strategy for chemical inhibition of BCL-2 survival proteins [8].

A second (potentially insurmountable) barrier for rational development of BCL-2 inhibitors is the lack of an established molecular function for these survival proteins. The large number of possible proapoptotic binding partners for BCL-2 has been progressively whittled away, following the recognition that heterodimerization could be induced by detergent [9]. Alternative modes of action have also been proposed involving regulation of different aspects of mitochondrial homeostasis [10]. In place of a well-defined functional assay, several strategies have been used to demonstrate the intracellular activity of candidate molecules against BCL-2 survival proteins, e.g., FRET analysis of associations with proapoptotic proteins and generation of mutant BCL-X_L proteins resistant to small molecule inhibitors [6, 11].

Kitada and colleagues identified gossypol as a BCL-X_L inhibitor using a fluorescence polarization assay for displacement of a labeled BAD BH3 peptide [12]. Direct evidence of gossypol binding to BCL-X_L was obtained using two complementary NMR spectroscopy techniques. One-dimensional diffusion-edited ^1H spectra of gossypol in the presence and absence of protein were consistent with low to submicromolar binding affinities. Chemical shift mapping of [^{15}N,^1H]-TROSY HSQC spectra showed broadening and loss of >1/3 of the resonance peaks with gossypol binding, including the BH3 binding cleft. These results suggest that the protein conformation changes with gossypol binding, as observed for the cleft-opening BH3 peptides [13].

A docking model of gossypol in the BCL-X_L hydrophobic groove suggested that the reactive aldehyde functional groups could be removed without changing the binding configuration. Direct comparison of apogossypol showed a moderate decrease in BCL-X_L binding affinity (Ki 2.3 μM versus 0.3 μM) but similar cytotoxicity profile compared with the parent compound. While binding assays are performed with soluble BCL-X_L, it is important to remember that BCL-X_L requires association with lipid membranes for antiapoptotic function, and the membrane-inserted conformation has not been structurally characterized. Among the biological activities reported for gossypol, several (inhibition of lactate dehydrogenase, erythrocyte anion transport, and spermicidal activity) are deficient in apogossypol. More extensive structure-activity relationships will need to be developed to assess the specificity of apogossypol as a BCL-X_L inhibitor.

Beccatini and colleagues provide a window on the intracellular effects of apogossypol by observing rapid loss of mitochondrial localization of soluble proapoptotic BCL-G(S) protein fused to green fluorescent protein. This effect was not seen with expression of a mu-

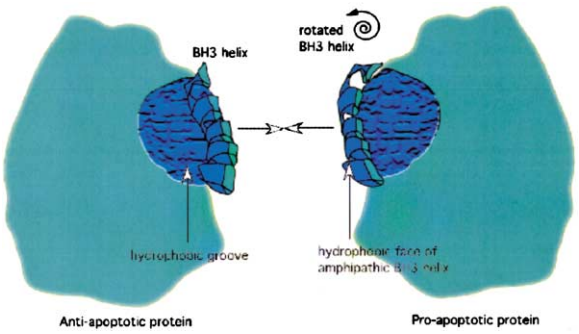

Figure 1. Model of Dimer Interaction between Anti- and Proapoptotic BCL-2 Family Members

tant BCL-X_L protein that has reduced affinity for gossypol, consistent with the previous demonstration of BH3-dependent association of BCL-G(S) with BCL-X_L. Cytotoxic activity of apogossypol is also demonstrated against chronic lymphocytic leukemia (CLL) cells derived from untreated and treatment-refractory patients. As CLL is most commonly associated with high expression levels of BCL-2, it is probable that apogossypol inhibits both BCL-X_L and BCL-2. The current generation of BCL-2 inhibitors appear to have similar binding affinities for BCL-2 and BCL-X_L, suggesting that conserved features of the binding pockets are being sampled. An important test of the static and dynamic features of the binding models will be the development of inhibitors with greater specificity for individual survival proteins.

With several BCL-X_L/BCL-2 antagonists now available [14], important questions for the functions of these proteins can be better addressed. Are proapoptotic members required for the lethal effects of BCL-2 inhibition? What are their effects on isolated mitochondria? Is there more than one class of chemical inhibitors? Different pharmacophores? Is chemical inhibition different from acute loss of expression (using antisense or RNAi methods)? The answers to these and other questions are eagerly awaited.

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Nano-Tailoring: Stitching Alterations on Viral Coats

Growing interest in utilizing protein assemblies for nanomaterials applications has spawned efforts to customize these scaffolds. Viral capsids have been modified with new chemical functionalities, typically at lysine or cysteine residues. Two innovative studies describe approaches to introduce modifications at virion tyrosine residues [1, 2].

The quest to create new nanometer-sized chemical architectures continues to accelerate, primarily along three broad trajectories: (1) top-down designs, using microlithographic and other techniques to embed increasingly smaller features into macroscopic materials; (2) bottom-up designs, using the techniques of supramolecular chemistry to control the self-assembly of multiple constituent molecules into well-defined complexes; and (3), nanoscale redesigns, utilizing existent biogenic assemblies of the proper dimension and altering their chemical composition to attain a desired structure and/or function.

Viruses are intrinsically attractive scaffolds for nanoscale constructions because they are predisposed for self-assembly to form highly stable symmetrical structures with dimensions in the tens of nanometers. The detailed three-dimensional structures are often known from X-ray crystallography, and it is possible to introduce specific positions of chemical reactivity on viral proteins through standard site-directed mutagenesis protocols. Virions can even be produced cost effectively on the gram scale. Self-assembly does not end at the level of the individual capsid; in many cases, the viral particles can be readily crystallized, even after modification, leading to organization of nanoblock arrays on the millimeter scale. A number of research groups are rapidly expanding the repertoire for nanochemistry on biological scaffolds [3]. Substantial effort has been devoted to utilizing biopolymers as templates for organized formation of inorganic materials [4, 5]. Recently, progress