Using Chemical Biology to Target the Inhibitors of Apoptosis

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The inhibitors of apoptosis (IAP) are a group of eight human proteins thought to inhibit apoptosis (programmed cell death), promoting cellular immortality characteristic of cancer cells. The IAPs, which include XIAP and survivin, share one or more baculoviral IAP repeat (BIR) domains. The third BIR domain of XIAP, for which an inhibitor exists, was used as a control in identifying small-molecule modulators of survivin, for which none exists. A method to prepare the XIAP BIR3 domain for both one-bead-one-compound and functional chromatography natural product screening was developed that is applicable to all IAPs. BIR3 was cloned into DH5a E. coli cells, expressed in BL21(DE3) E. coli cells, and purified with ion metal affinity chromatography. An unnatural amino acid with a bio-orthogonal chemical handle was site-specifically incorporated into BIR3, however cysteine-based fluorescent tagging was pursued on a large-scale. A glutathione-agarose-based binding assay has been designed to determine functionality of labeled BIR3 based on binding to GST-tagged caspase-9. BIR3 and survivin were linked to a solid support to carry out a functional chromatographic screen with four natural product extracts and several unique peaks were identified for further analysis. Conducting a one-bead-one-compound screen of BIR3 will serve as a control to gauge the potential success of an eventual screen against survivin. Additional natural product extracts will be screened against BIR3 and survivin to identify potential binders. Small-molecule hits from both screens will be confirmed with analytical chemistry, biochemical, and biophysical investigations and evaluated as cancer therapeutics that disable survivin and the other IAPs.

Awards Won: