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Molecular Staples Shape a Cancer Killer

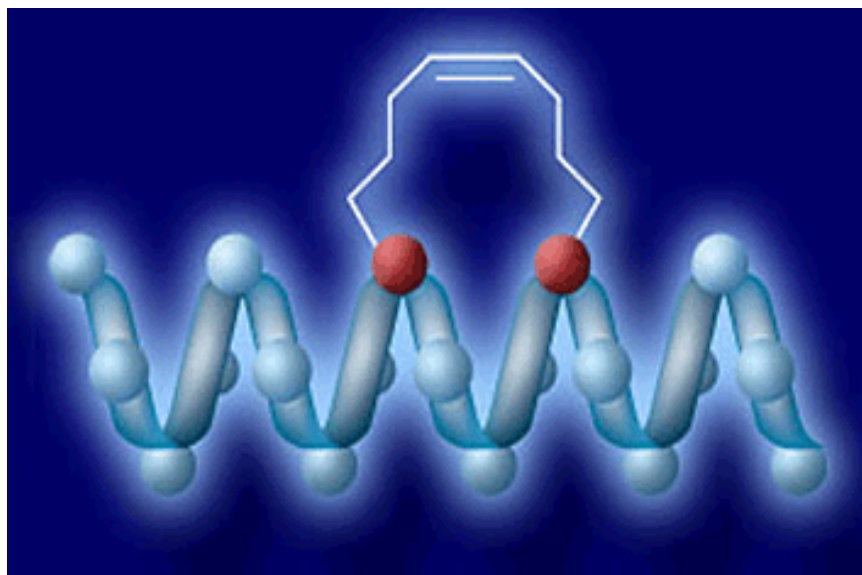


Image Title: - Eric D. Smith, Korsmeyer Laboratory

Howard Hughes Medical Institute (HHMI) researchers have successfully designed and improved a new type of cancer-killing compound by performing molecular surgery to stabilize the molecule so that it selectively triggers cell death.

The idea for developing the compound emerged from the HHMI laboratory of Stanley J. Korsmeyer, who leads one of the hottest research teams currently studying programmed cell death, or apoptosis, a genetic program that executes cells that are no longer needed. Using the biologically active portion of a protein that triggers apoptosis, Korsmeyer's team successfully inserted non-natural amino acids into the peptide sequence and then performed a chemical reaction that created a "staple" within the molecule, resulting in its stabilization. Korsmeyer and the paper's lead author, Loren D. Walensky, who are at the Dana-Farber Cancer Institute at Harvard Medical School, reported their studies in the September 3, 2004, issue of the journal *Science*.

The chemical approach they applied, called hydrocarbon stapling, was developed by their collaborator Gregory L. Verdine of Harvard University, and permitted the researchers to overcome the tendency of short peptides to lose their critical three-dimensional structure - and their ability to kill cells—when removed from the context of the complete protein. This has been one of the greatest obstacles associated with using short peptides as therapeutic agents, and has hindered their legitimacy as pharmaceutical lead compounds. By making the peptides more resistant to degradation and enabling their cellular uptake, the hydrocarbon staple overcomes classic shortcomings of peptide therapeutics.

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- Loren Walensky

As the molecular events that lead to cell death have become clearer during the last decade, researchers speculated that it would not be long before biotechnology and pharmaceutical companies raced to develop novel compounds that could be used to hasten or prevent the demise of cells.

“Academic and industrial laboratories are engaged in a Herculean effort to develop new molecules that reactivate the apoptotic program in tumor cells,” wrote HHMI investigator Steven F. Dowdy of the University of California, San Diego, in a *Perspectives* article that was published in the same issue of *Science*.

Korsmeyer, Walensky, and their colleagues aimed to construct a key regulatory segment of an apoptosis-triggering protein called BID that could induce apoptosis in cancer cells. Their objective was to create a short peptide that functionally mimicked the specific region of the BID protein that elicits cell death. Theoretically, such a small molecule—basically a short string of amino acids—could insinuate itself into cancer cells to trigger their suicide.

“Our goal was to modify the natural peptide sequence only enough to stabilize or reinforce its shape to improve its pharmacological properties,” said Walensky. The researchers used the hydrocarbon stapling strategy to brace the peptide from within.

“We substituted non-natural amino acids for natural amino acids in selected positions,” he said. “The non-natural amino acids look very similar to the natural ones, except that they include hydrocarbons that can be cross-linked to one another. This cross-linking provides a constraint, which doesn't allow the peptide to unfold.” The researchers dubbed the engineered peptide “stabilized alpha-helix of BCL-2 domains” (SAHB). The BCL-2 family of

proteins regulates apoptosis.

When the researchers examined SAHB's properties, they found that it assumed a stable alpha-helical shape, bound to the right protein to trigger apoptosis, and resisted degradation by proteases. They also found that SAHB specifically triggered the cell's power plants, the mitochondria, to release a protein that participates in launching apoptotic destruction.

Their experiments with leukemia cells cultured in the laboratory revealed that SAHB could enter, and, more importantly, inhibit the growth of those cells.

“When we saw that we could activate cell death in a specific way in whole cells, we were eager to conduct animal studies,” said Walensky. So, the researchers tested the effects of SAHB on mice that harbored human leukemia cells. “We found that SAHB treatment effectively suppressed leukemia in these mice,” he said.

According to Walensky, the experiments are the first steps in a broader effort to construct hydrocarbon-stapled alpha-helical peptides that affect many control points of apoptosis. These are necessary because different cancers may have thwarted the apoptotic machinery in different ways, he said.

“The goal would be to use the natural sequences of these pro-apoptotic peptides to try to specifically activate the cell death program in a resistant cell,” said Walensky. “For example, certain lymphomas are specifically driven by BCL-2 overexpression. So, if you could knock down some of the impact of that BCL-2, you would tip that lymphoma cell over the edge toward death.”

Korsmeyer, Walensky, and their colleagues are now developing and testing different hydrocarbon-stapled BH3 domains against a range of cancer cells. Ultimately, they believe that further development of these peptides could broaden the arsenal of compounds used to kill different cancers.

Walensky said he would not be surprised to see hydrocarbon stapling of alpha-helices applied to control many interactions between proteins. “The alpha-helix plays a pivotal role in many biological interactions,” said Walensky. “So if we could target protein-protein interactions at critical biological control points using the natural, evolutionarily derived sequence for that protein target—with just this minor modification of hydrocarbon stapling—then we might have a whole new set of tools to study and manipulate protein interactions within cells.”