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Smac-ing Back at Cancer Cells

By mimicking a molecular switch that triggers cell death, researchers have killed cells grown in the laboratory from one of the most resilient and aggressive cancers - a virulent brain cancer known as glioblastoma. The new approach to tricking the cell-death machinery could be applied to a wide range of cancers where this pathway, known as apoptosis, has been inactivated.

The researchers—led by Xiaodong Wang, a Howard Hughes Medical Institute investigator at the University of Texas Southwestern Medical Center at Dallas and his colleagues Patrick Harran and Jef De Brabander—published their findings in the September 3, 2004, issue of the journal *Science*.

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Many cancer cells are particularly hardy because they have switched off the apoptotic machinery at one point or another, protecting them from the suicide process that their aberrant behavior would otherwise trigger.

To reactivate the cell-death pathway in cancer cells, the researchers sought to create a molecular mimic of a protein called Smac, which promotes apoptosis. Normally, when apoptosis is activated in cells that are damaged or no longer needed, Smac is released from the mitochondria, which are the cell's power plants. Once released, Smac binds to a group of gatekeeper proteins known as "inhibitor of apoptosis proteins" (IAPs), which normally hold in check the cell's chief executioner enzymes. These enzymes, called caspases, wreak lethal havoc in cells targeted for apoptosis. Smac's action is why it was named the "second mitochondria-derived activator of caspases."

Specifically, Wang and his colleagues sought to make a small molecule to mimic the function of the Smac protein, since a smaller molecule is better able to pass through the cell membrane to reach the cell's interior, where IAP-caspase resides.

"The idea for making this inhibitor molecule first arose in previous studies when our collaborator Dr. Yigong Shi solved the crystal structure of Smac

interacting with the target protein IAP,” said Wang. “We realized that the interactive motif of Smac with that protein is only four amino acids, so it was possible to make a small-molecule mimic.”

According to HHMI investigator Steven F. Dowdy, who co-authored a *Perspectives* article in *Science*, the key to the study is that Wang and his colleagues took advantage of the fact that the Smac-IAP protein-protein interaction is relatively unstructured, since only the N-terminal amino acids of Smac interact with IAP.

“This property allowed them to readily create a library of non-natural amino acids and search for one that looked like the Smac N-terminal domain and fit into the groove of IAP that triggers it to unleash caspases. And they found one that responds nearly identically in terms of concentration, but it's resistant to proteases and it can penetrate the cell membrane just like other small molecules,” said Dowdy, who is at the University of California, San Diego School of Medicine. The result of the search for Smac mimics was a molecule the researchers called “Compound 3.”

“The way we arrived at Compound 3 was serendipitous,” said Wang. “At first we thought that just mimicking the last four amino acids of Smac was the way to go, but we weren't getting anywhere. But in one of the chemical reactions, we actually made a dimer - linking the molecules in pairs. That dimer, Compound 3, turned out to be much more active.” The scientists believe the twinned molecule is more active because the Smac protein itself is a combination of two identical proteins, although the reason for Compound 3's activity remains unclear.

Compound 3's striking apoptosis-triggering activity revealed itself when the scientists introduced it into cultures of human glioblastoma cells. “We picked human glioblastoma because it is the hardest to kill,” said Wang. “The cells grow like weeds and they are tough as a rock.”

The cells, however, were no match for Compound 3. When the researchers added the Smac mimic to glioblastoma cultures—along with a protein called TRAIL that also helps activate the apoptosis machinery—it easily killed the cells. In contrast, they found, the same treatment had no effect on normal human fibroblast cells.

“One particularly important finding is that the compound is effective at extremely low concentrations, already below those necessary for other commonly used anti-cancer drugs to work,” said Wang. The low dosage needed to kill the cells suggests that as a therapy, the molecule may have fewer non-specific toxic side effects than many anti-cancer drugs.

IAP is also involved in another apoptotic process — that triggered by a receptor protein called TNF alpha, which also triggers the inflammation process. The researchers found that Compound 3 also switched on apoptosis in cells treated with TNF alpha. Thus, said Wang, Compound 3 might also be used as an anti-inflammatory drug.

“Although this is still a hypothesis, it might be that, for example in rheumatoid arthritis, if we treated with something like Compound 3, it would cause TNF alpha to trigger apoptosis in the cells that cause joint and tissue damage. Thus, the secondary inflammation from these cells would be prevented.”

However, he said, further studies in his laboratory will concentrate mainly on using Compound 3 as a prototype treatment for cancers. The researchers are currently testing the molecule's effects on an array of cultured cancer cells. They also plan to begin testing the compound in animal models of cancer, to explore its effectiveness, stability and distribution *in vivo* .