

Two Roads to Cell Death

Can cancer cells be reset to respond to suicide signals?

Small protein segments created by HHMI investigator Stanley J. Korsmeyer, Anthony Letai and their colleagues at the Dana-Farber Cancer Institute and Harvard Medical School have revealed important new details about the intricate machinery of cell suicide. The work, reported in the September 2002 issue of *Cancer Cell*, reveals two distinct pathways to trigger cell death and points the way to drugs that stimulate the process in cancer cells.

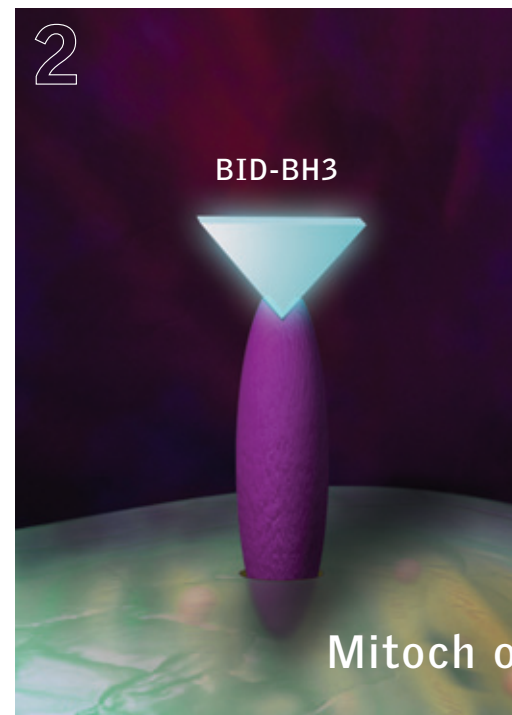
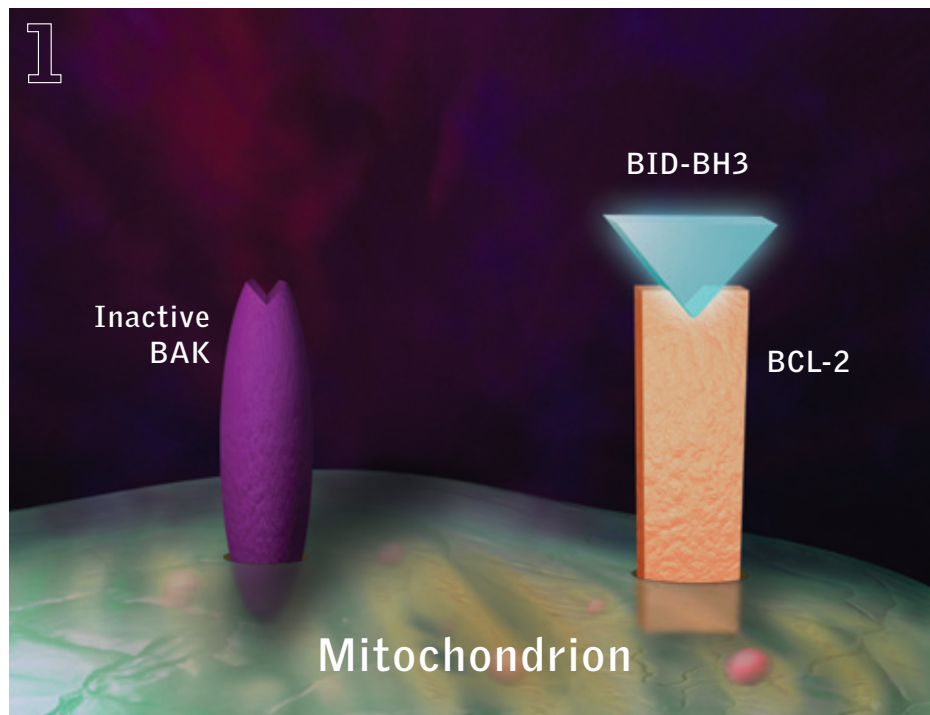
Cells that are defective or that become unnecessary during growth and development are induced to commit suicide through a finely balanced process known as apoptosis. Certain proteins activate this cellular suicide, while other proteins protect against it. If apoptosis is disabled, the body loses its natural protection against cancer, and cells grow out of control.

The scientists concentrated on proteins containing only the BH3 domain, the prodeath component of apoptosis. These proteins are offset by anti-apoptotic proteins, notably the protective protein BCL-2. Although the various proteins with BH3 domains have some similarities in structure, the differences among them are so significant

that the proteins could play many roles in apoptosis, says Korsmeyer. To attempt to delineate those roles, the scientists decided to construct peptides representing only the segments known to be BH3 domains.

“Our major question up front was whether the small death domains of these proteins themselves actually do anything,” says Korsmeyer. “These apoptotic proteins are pretty large, so it was seen as a bit of a stretch to suppose that just the BH3 domains would be functional.”

The true test of the synthesized BH3 domains would be whether they triggered cell death, either in mitochondria (the structures in the cell where the destructive process begins) or in whole cells. The peptides’ mechanism of action would be revealed most clearly in mitochondria; however, an ability to activate apoptosis in whole cells would suggest that the peptides could be prototypes of anticancer drugs. So the researchers tested their BH3 peptides in genetically altered whole cells and in their isolated mitochondria that either lacked certain components required for apoptosis or that overexpressed the protective protein BCL-2.



Their experiments revealed clearly for the first time that BH3 domains from different cell-suicide proteins govern two distinct mechanisms for triggering apoptosis:

- BH3 domains from the proteins called BID and BIM trigger apoptosis directly, by activating other proteins that release the death-triggering substance cytochrome *c* from mitochondria.
- BH3 domains from the proteins called BAD and BIK trigger apoptosis indirectly, by binding to and interfering with the protective protein BCL-2. (Normally, BCL-2 prevents cell death by binding the BH3 domains that trigger apoptosis directly. Those domains become free to act when the BH3 domains from BAD and BIK bind to BCL-2.)

The scientists also studied the cancer-killing potential of BH3 domains. They introduced the BH3 peptides into cultures of

leukemia cells and found that the peptides could activate the indirect apoptosis pathway to kill the cells.

“These findings have led us to view these BH3 domain peptides as highly instructive tools to understand apoptosis,” Korsmeyer says. “And while they will not themselves prove to be anticancer compounds, they are important prototypic leads to development of such drugs. Tony Letai has laid out a pathway for pharmaceutical companies to begin to screen small molecules for such activity.”

Drugs specific for tumors would most likely be peptide mimics that use the indirect pathway to induce suicide in cancer cells, according to Korsmeyer. “We are most excited by the BAD-like compounds that would sensitize cancer cells to apoptosis,” he says. “If cancer cells—one example being leukemia—are protected by anti-apoptotics like BCL-2, a BAD-like drug could tie up the BCL-2 and reset the sensitivity of the apoptotic pathway. Then, you could hit the cancer cells with a second drug that is tumor cell specific and that would trigger apoptosis only in those cells.”

—DENNIS MEREDITH

INDIRECT KILLING

1. Cancer cells are protected from committing suicide, or apoptosis, by a protein called BCL-2 on the surface of the cell's mitochondrion. BCL-2 grabs hold of a peptide called BID-BH3 and keeps it from doing its job, which is directing the cell to die. With BID-BH3 out of action, the cancer cells go on living, and multiplying.

2. When another peptide called BAD-BH3 is added, it binds to BCL-2, and the cell can once again undergo apoptosis. BCL-2 can no longer bind BID-BH3, leaving BID-BH3 free to activate a protein, called BAK, on the mitochondrion's surface.

3. Once BAK is activated, cytochrome *c* is released from the mitochondrion and the cell dies. Korsmeyer and colleagues hope that a drug like BAD-BH3 will be developed to reset the susceptibility of cancer cells to die. A second agent that activates a BID-like BH3 in the cancer cell holds the promise of selectively killing that cell.

