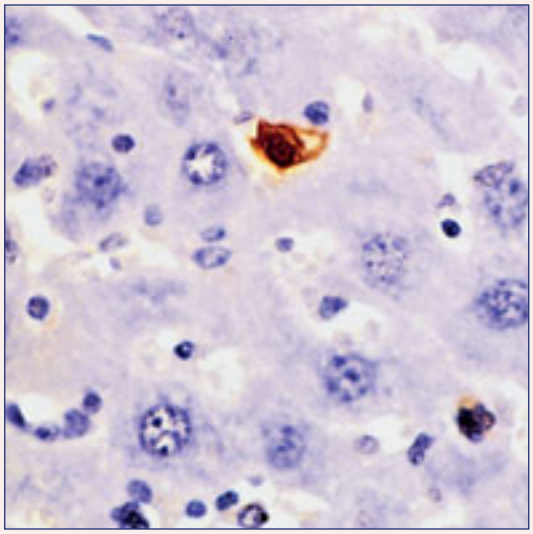
AIMING AT APOPTOSIS

The cell-suicide pathway is a major target for research into new cancer drugs.

BY RANDALL C. WILLIS AND MARK S. LESNEY

NEARLY EVERYONE IS AWARE THAT DEATH is a way of life in the development and protection of multicellular organisms. Cells die by the millions to perform a myriad of necessary functions, from the carving out of body cavities to the sculpting of organ shapes. But with apoptosis, cells do not make the decision to die willy-nilly by themselves. They receive the message to disintegrate—not so much a voluntary decision as the ultimate surrender to metabolic peer pressure from the cells around them and the body that used to be their nurturing home.

But sometimes cells transform, and rather than going gentle into that good night, they become willful traitors whose self-interest clutches at life at all cost, refusing to die for the greater good, greedily reproducing their kind to the point that metastasizing tumors or flooding leukemias doom the entire body in a mass-murder/suicide pact.



The failure of apoptosis to destroy cells in the process of transforming from Dr. Jekylls to ravening Mr. Hydes yearly kills more than 500,000 people in the United States alone. If the message to die could be made more persuasive, if the rebel Hydes could be singled out among the willing-to-die Jekylls and convinced to do their duty and depart, then, the theory goes, many cancers could be vanquished—without simultaneously destroying the body that houses them. Numerous current cancer chemotherapies work upon this principle—though with generally less-than-perfect effect.

Understanding the cell death signaling process and its internal manifestations is the first step to designing better drugs to activate it, in the case of cancer, or stop it, in the case of osteoarthritis, neurodegenerative diseases, Type I diabetes, and hyperreactions to infection or trauma, which are the result of cells responding with excessive apoptotic zeal. The following briefly outlines some of the key processes of apoptosis as illustrated in the poster on the next two pages, and in doing so, points to where new drugs could more effectively modulate cellular self-destruction.

THE CASPASE CASCADE

All apoptosis proceeds through the action of the various caspases, a family of cysteine-aspartyl-specific proteases that induce cell death by cleaving adjacent to aspartic acid residues in substrate proteins that affect the cellular chromatin, cytoskeleton, and nuclear envelope. Eleven caspase-encoding genes are currently identified in the human genome (*1*).

Cells dying from this process maintain plasma membrane integrity. And although they show evidence of shrinkage and nuclear collapse, they do not simply die, but are finally "killed off" by being devoured by phagocytes that recognize their distress and administer the coup de grâce—thereby preventing the release of damaging inflammation-causing compounds.

Because apoptosis is both so "final" and so important, it is multiply regulated. Inactive caspases (zymogens) exist in a pool that must be activated through proteolysis by specific caspase-activating proteins, many of which are triggered by signals from outside the cell. Normally, the activated caspases are further held under

PHOTO: CENTERS FOR DISEASE CONTROL AND PREVENTION

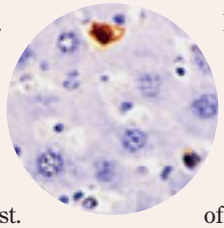
Table 1

Companies participating in the search for apoptosis-related drugs

	Company	URL
Pro-apototic compounds	Applied Molecular Evolution/MedImmune	www.amevolution.com/Pipeline/vitaxin1.htm
	Aton Pharma	www.atonpharma.com/saha.cfm
	Gemin X Biotechnologies	www.geminx.com
	Genentech	www.gene.com
	Genta	www.genta.com/genta/Products/genasense.html
	Introgen Therapeutics	www.introgen.com
	National Cancer Institute	www.cancer.gov
	Onyx Pharmaceuticals	www.onyx-pharm.com/products/onyx_015.html
	OSI Pharmaceuticals	www.osip.com/programs
	SuperGen	www.supergen.com
	Targeted Genetics	www.targetedgenetics.com
Anti-apoptotic compounds	Aventis	www.aventisoncology.com
	Forest Laboratories	www.frx.com
	Idun Pharmaceuticals	www.idun.com
	Novartis	www.novartis.com

negative control by inhibitors of apoptosis proteins (IAPs). These suppressors have a zinc-binding fold that enables them to attach to and inhibit activated caspases.

In many cancer cells, a number of these IAPs are overexpressed and protect against apoptosis. One such protein, called survivin, has been identified by comparing the protein expression profiles of normal and cancer cells. Survivin "represents one of the most cancer-specific genes in the entire genome" (*1*). Obviously, IAPs such as survivin are potential targets for drugs to promote apoptosis of cancer cells.



Signals to suicide

To obtain the proper death-triggering information from outside the cell, several methods of signal reception exist. The most important of these are the cytokine tumor necrosis factor (TNF) plasma membrane receptors. Members of this family bind TNF1, FAS, and TRAIL cytokines. By activating intracellular procaspase-binding proteins that contain cytosolic death domains, these receptors ultimately trigger the activation of the caspases and the apoptotic process. In a more intimate apoptosis-inducing process, cytotoxic T lymphocytes and natural killer (NK) cells inject proteases such as granzyme B through perforin channels leading into the cells marked for death. Granzyme B activates caspases, leading to apoptosis.

Mitochondrial menace

One of the most significant gene families associated with apoptosis is *BCL2*, which includes 25 proteins, many of which interact as homo- or heterodimers to enhance or suppress apoptosis in the presence of various apoptotic stimuli, including anticancer drugs. The BCL2 proteins primarily act by regulating the release of proteins from mitochondria. Pro-apoptotic BCL2s stimulate the release of cytochrome c, which binds and activates APAFI, one of a family of 20 caspase-activating proteolytic proteins. In turn, APAFI binds and activates, through cleavage, the zymogen procaspase 9, which as active caspase 9 promotes apoptosis.

Other directions

Besides these direct ways of interacting with the apoptotic pathway, a host of other possible means is available for modulating the various input factors and signaling pathways that trigger the process. All of these, from mitogen-activating protein (MAP) kinases to phosphatases to transcription factors, are also being studied in hopes of finding new drugs to modulate this most important life-or-death struggle.

Reference

(1) Reed, J. C. *Nat. Rev. Drug Discov.* **2002**, *1,* 111–121.

Further reading

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Randall C. Willis and **Mark S. Lesney** are senior associate editors of *Modern Drug Discovery*. Send your comments or questions about this article to mdd@acs.org or to the Editorial Office address on page 3. ■

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