Cell Death Studies Yield Cancer Clues

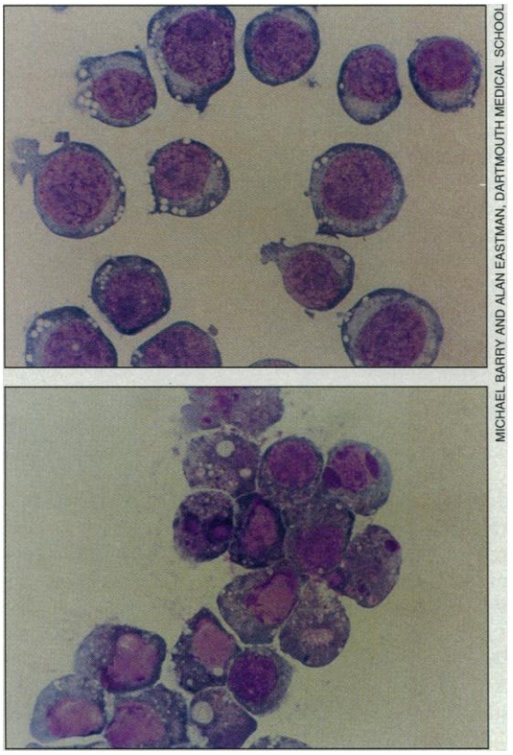
The realization that malignant tumors may grow because cells don't die when they should is providing a new direction for both basic and clinical cancer research

Much as we might dislike the idea, death is a natural part of life. And that ineluctable fact is just as applicable to individual cells as it is to entire organisms. If cells don't die at the right times and in the right places, the developing brain can't form its myriad precise connections between nerve cells (see accompanying story on page 762). Nor would the immune system be able to rid itself of cells that attack the body's own tissues. And now, it seems, cancer may be still another consequence of cells' failure to die when they should.

This conclusion, coming from recent work in numerous laboratories around the world, is providing an entirely new direction for cancer research. Only a few years ago, researchers trying to understand what causes malignant tumors to grow focused almost all their attention on the pathways within cells that tell them when to divide, figuring that in cancer they've gone haywire, causing uncontrolled cell proliferation. But the new work is showing that cells also have internal pathways that tell them when to die. And in cancer, the death pathways may be suppressed—extending the cells' lives while putting the bearer in danger of death.

Robert Horvitz of the Massachusetts Institute of Technology (MIT) is working out the pathways of cell death in the roundworm *Caenorhabditis elegans*. At a meeting* last fall on the "Genetics of Cancer," he pointed out: "Tissue homeostasis is essentially a balance between [cell] proliferation and death, and too much growth can come from too little death as well as from too much proliferation." This realization may have important consequences: Learning how death pathways work may help drug designers develop more effective tumor-shrinking agents—say, ones that revive the suppressed death pathways.

While cancer researchers have become interested in cell death only recently, the topic itself is far from new. Developmental biologists have known for decades that many cells must die in order for an embryo to mature normally—that, in fact, certain cells are programmed to die at certain points during development. Then came John Kerr at the University of Queensland School of Medicine in Australia and Andrew Wyllie at University Medical School in Edinburgh, Scotland. About two decades ago, they described a set of characteristic changes that make cells dying by programmed death readily identifiable: The cells shrink; their chromatin (the complex of DNA and proteins in the nucleus) condenses; and their membranes develop protuberances called "blebs." Kerr and Wyllie's work raised the profile of programmed cell death in two other ways as well. They noted that it is not limited to embryonic development. And they gave it an unusual new name—"apoptosis," from a Greek word describing a flower losing its petals or a tree its leaves.



MICHAEL BARRY AND ALAN EASTMAN, DARTMOUTH MEDICAL SCHOOL

Programmed to die. Leukemia cells, treated with a drug, show classic signs of apoptosis *(bottom)*.

Since then, numerous researchers have turned up examples in which cells undergo apoptosis, which can be triggered by a variety of changes in their environment. Among them: Prostate cells are programmed to die when deprived of male hormones. And certain immune cells go into apoptosis when deprived of cytokines such as interleukin 2. The fact that cells can respond to hormone deprivation and other changes in their environment by undergoing programmed cell death was one indication that it's regulated by the cell, as was the discovery that it often requires active protein synthesis.

The cancer connection

But interesting as all those findings were to cell biologists, apoptosis didn't have much impact on the cancer community until the late 1980s when researchers, including Suzanne Cory's team at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, and Stanley Korsmeyer's at Washington University School of Medicine in St. Louis, connected it to an oncogene known as *bcl-2*. This oncogene had been discovered a few years earlier in lymphoma tumors composed of B cells. The supposition was that its activation contributed in some way to the tumor development, but no one knew how.

What the Cory and Korsmeyer groups showed is that if they introduced *bcl-2* into B cells and then cut off their cytokine supply, the cells survived—even though they were supposed to go into apoptosis and die when deprived of cytokines. At first, this work was limited to cultured cells. But the researchers made transgenic mice bearing the *bcl-2* oncogene and showed that it extends B cell survival in living animals, too. So it looked as if *bcl-2* was a kind of antidote to apoptosis—and that suggested a way it might contribute to cancer development.

Further work showed that *bcl-2* can inhibit apoptosis induced by several other stimuli in addition to cytokine deprivation. "It's quite prolific in blocking programmed cell death," says David Hockenbery, who formerly worked in Korsmeyer's lab on the *bcl-2* project. And that's significant, he adds, because it seems to put *bcl-2* at a central point in the apoptosis pathways.

What everyone wants to know now is what it's doing there. At present, nothing is known about the function of the *bcl-2* protein, which has been detected in the mitochondrial membrane and also in the nuclear envelope and endoplasmic reticulum. One recent development that may help clarify *bcl-2*'s function

*The meeting, which was held in Hilton Head, South Carolina, on 4 to 8 November, was a Laureates Conference of the General Motors Cancer Research Foundation sponsored by the American Association for Cancer Research.

comes from Horvitz's group, however. The MIT workers have identified a gene called *ced-9*, which appears to be the *C. elegans* equivalent of *bcl-2*. The roundworm is much more amenable to the experiments needed to pin down the gene's function than are mammals such as mice.

While researchers hunt for that elusive piece of the puzzle, however, they've recently homed in on another—an oncogene called *myc*. Prolific as the *bcl-2* gene is in its ability to inhibit apoptosis, it can't make cells cancerous on its own. And that's where *myc* comes in: It can apparently cooperate with *bcl-2* in inducing tumors.

Researchers have known for several years that *myc* activity stimulates cell proliferation, but in the past year or two, three groups, led by Gerard Evan of the Imperial Cancer Research Fund in London, John Cleveland of St. Jude Children's Research Hospital in Memphis, Tennessee, and Douglas Green of the La Jolla Institute of Allergy and Immunology, found that it can also play a counter-intuitive role: It can stimulate apoptosis as well. The protein made by *myc* essentially gives the cell two options, says Green: proliferate or die. Which of those two actually happens depends on what other signals the cell receives. If the cell receives additional survival stimuli, proliferation wins out. If not, apoptosis predominates. And *bcl-2*, the Green and Evans teams discovered last year, can provide the requisite—and disastrous—survival signal. Indeed, the Cory group had shown earlier that transgenic animals carrying both *bcl-2* and *myc* get B cell tumors much faster than animals that carry only *myc*.

Role for suppressor genes, too?

But the oncogene connection is only part of the reason why apoptosis research is attracting so much attention. These days, research on the tumor suppressor genes is perhaps, if anything, even hotter than research on oncogenes—and suppressors may also be participating in apoptosis regulation. Take the well-known *p53* suppressor gene. Cancer researchers have found that cells of a wide variety of tumors, including such common ones as breast and colon cancer, have either lost the gene, or have a mutated version. The supposition is that protein made by the normal *p53* gene acts like a brake on tumor growth. Now there are hints it might do this by bringing about apoptosis.

About a year and a half ago, a team including Moshe Oren, Leo Sachs, and their colleagues at the Weizmann Institute of Science in Rehovot, Israel, introduced a normal *p53* gene into leukemia cells lacking the gene. The result: the cells underwent apoptosis. What's more, a mutation in the gene may cripple its ability to trigger apoptosis. Sachs and Joseph Lotem, who's also at Weizmann, have found that a mutant version of the *p53* gene acts much like *bcl-2* in blocking apoptosis induced by *myc*. And *p53*'s effects aren't limited to leukemia cells. Philip Shaw's group at the Institutes of Pathology and Microbiology in Lausanne, Switzerland, showed that restoration of *p53* gene activity in colon cancer cells also induces apoptosis.

Looking to the future

Even though researchers still have a long way to go before they fully understand apoptosis pathways, some scientists are plunging ahead to answer the next big question: Can stimulation of apoptosis be used clinically to cause tumor regression? And even this early in the game, there is preliminary evidence that it may.

Some Genes Implicated in Apoptosis Control

Gene	Cellular Location of Protein Product	Effect on Apoptosis
bcl-2	Mitochondrial membrane Nuclear envelope Endoplasmic reticulum	Blocks
myc	Nucleus	Stimulates (also stimulates cell proliferation)
p53	Nucleus	Wild type stimulates Mutant blocks
APO-1/Fas	Cell membrane	Stimulates

Shaw and his colleagues have found, for example, that *p53* activation can cause the shrinkage of experimental colon tumors in animals. And Peter Krammer's group at the German Cancer Research Center in Heidelberg has another potentially promising result. A few years ago, the team identified a monoclonal antibody that very efficiently triggers apoptosis in leukemia cells in culture. They subsequently showed that the antibody, which recognizes a cell surface molecule called APO-1, also induces the regression of experimental tumors in mice. After one shot of antibody, "large tumors are gone in about a week," Krammer says. (APO-1 was also identified by a group led by Shin Yonehara of the Tokyo Institute of Medical Science, who gave it the name Fas.)

And there's more reason for hope. Apoptosis work may also shed some light on one of the thorniest problems in cancer therapy—the development of resistance to chemotherapeutic drugs. Often these drugs work for a time but lose their effectiveness as cells devise ways to circumvent their effects. Enter cell biologist Alan Eastman of the Dartmouth Medical School and his colleagues, who have found that several chemotherapeutic drugs work by inducing apoptosis. "We screened about 10 drugs and always found the same thing," Eastman says. "Apoptosis is sort of an end product of a number of different pathways of cytotoxicity."

Eastman's finding raises the possibility that one way cells become resistant to chemotherapeutic drugs is by turning on genes such as *bcl-2* that block apoptosis or, conversely, by turning off genes such as *p53* that induce apoptosis. A finding that supports that idea comes from John Reed of the Cancer Research Institute in La Jolla. He's shown that putting the *bcl-2* gene into drug-sensitive cells makes them resistant. But it may nevertheless be possible to circumvent the blocks put up by inhibitory genes and find drugs that can activate apoptosis again, says cancer specialist John Isaacs of the Johns Hopkins University School of Medicine.

One example involves prostate cancer, which is often treated with drugs that block the action of male hormones because prostate cells go into apoptosis when deprived of the hormones. But because some of the cancer cells in the tumors have undergone changes that make them androgen-independent, and because these cells eventually take over, the drugs eventually lose their effectiveness.

But Isaacs and his colleagues have found that they can put even the androgen-independent cells into apoptosis with drugs that raise calcium ion concentrations within cells. They chose those drugs because Edinburgh's Wyllie has shown that fragmentation of the nuclear DNA is a very early event in apoptosis, perhaps even the ultimate trigger that gets the process under way, and a great deal of evidence suggests that this fragmentation is brought about by a DNA-splitting enzyme called an endonuclease that requires calcium ions for its action. The problem so far, Isaacs says, is that the drugs' effects aren't specific and they are too toxic for use in human patients. He is currently trying to reduce that toxicity by targeting them to prostate cells.

Of course, this is but one of many challenges that will have to be met by drug-designers trying to move apoptosis research into the clinic. Another is discovering the identity of the endonuclease enzyme since it would make a good target for therapeutic drugs. No surprise, several groups are working on identifying that enzyme—and even have candidates—but it's too early to tell which is likely to win out. With questions like these to be solved, excitement is running high in labs all over the world. You could put it this way: Research into cell death is showing more than a little life.

–Jean Marx