Oncogenes Evoke New Cancer Therapies

Research on the cancer-causing oncogenes has suggested several possible ways of blocking tumor cell growth; clinicians are beginning to put those ideas to the test

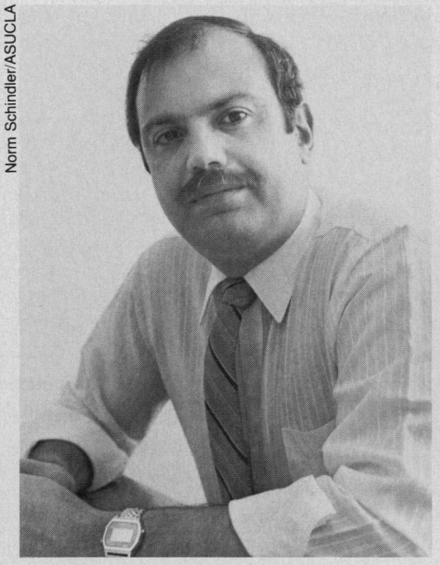
CANCER RESEARCHERS are about to begin testing a novel type of cancer therapy: a precise attack on cancer cells that aims to deprive them of proteins they need to divide and grow. These experimental therapies are the first clinical fruits of a decade and a half of intense research on oncogenes.

That research showed that these cancer-causing genes are aberrant forms of the genes that encode growth factors and other proteins that control cell growth and differentiation. So by studying cancer, the oncogene specialists found that they were also reaping a bounty of information about how cells work normally.

But the work also raised a fundamental question that couldn't be answered just by studying oncogene action in cultured cells or even in human cancer tissue: Could new cancer therapies be devised that work by blocking the activity of the growth factors and other proteins that oncogenes encode? Now researchers are poised to begin answering that question. Earlier this month, at a symposium on "The Origins of Human Cancer" held at Cold Spring Harbor Laboratory as part of that institution's centennial year celebrations, investigators from three labs reported that they have already begun, or are on the verge of beginning, clinical trials of experimental therapies that seek to work by countering the action of oncogenes, particularly those encoding certain growth factors and growth factor receptors.

Many oncogenes—roughly five dozen at this point—have been discovered. Their protein products, which are located in different parts of the cell, have diverse modes of action. Therapies might be directed at any of them, but the growth factors work right at the cell membrane, and that is one of the things that makes them a logical place to start trying to devise new cancer treatments.

"The growth factors and their receptors are popular targets for rational therapy because they're accessible. It might be harder to interfere with a nuclear protein," says Jim Battey of the National Institute of Neurological Disorders and Stroke, whose group has just cloned the receptor for the growth factor bombesin, which may contribute to the development of an aggressive form of lung cancer (also see box on p. 1377).



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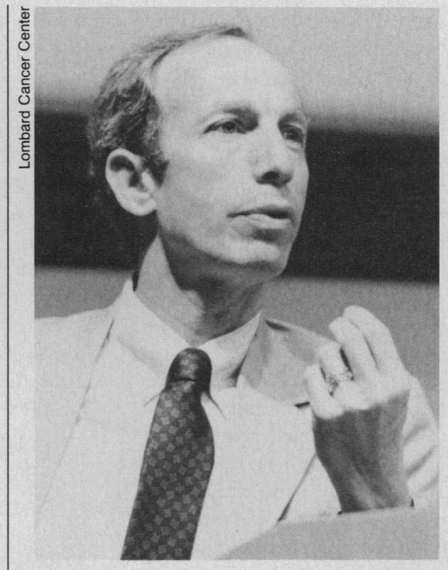


Lombard Cancer Center

Planning cancer trials. *Dennis Slamon (left) and Marc Lippman are gearing up to see whether blocking growth factor activity can help cancer patients.*

That's a rather practical reason for focusing on the growth factors as therapeutic targets, but there are sound scientific reasons as well. A great deal of evidence has shown that the cells of many cancers, including such common ones as breast and lung cancers, pour out growth factors. Work by Marc Lippman's group at Georgetown University School of Medicine has shown, for example, that breast cancer cells produce a long list of growth factors, including epidermal growth factor, platelet-derived growth factor, insulin-like growth factors I and II, one or more members of the fibroblast growth factor family, plus some growth-stimulatory agents that have not yet been characterized. At least some of these agents may contribute to the ability of the cancer cells to grow and invade new tissues.

If so, then blocking their activity might be a way of controlling cancer cell growth and in particular of preventing new tumors after the primary one has been removed surgically. The hope is that treatments directed at the growth factors or their receptors may be more specific than conventional radiation and drug therapies.

Although several oncogenes and growth factors have been implicated in the etiology of breast cancer, one of the most promising as a possible target for therapy is the oncogene variously known as *erb*B2, *HER2*, or *neu*. About 3 years ago Dennis Slamon and his colleagues at the University of California School of Medicine in Los Angeles reported that the *erb*B2 gene, which encodes a protein with all the hallmarks of a growth factor receptor, could be used to predict how breast cancer patients would fare. They had found that women whose tumor cells have extra copies of the oncogene were more likely to relapse and die than patients whose tumors did not have the gene amplification. The hypothesis was that having extra copies of *erb*B2, and therefore of the receptor it encodes, might enable tumor cells to grow and spread more aggressively than cells without the gene amplification.

The Slamon group's linkage of *erb*B2 gene amplification with poor breast cancer prognosis was controversial at first, as some researchers initially failed to confirm the correlation. But more recent—and much larger—studies conducted by several investigators, including Slamon, Lippman, and Adrian Harris of John Radcliffe Hospital in Oxford, England, have borne out the original conclusion. The newer work also indi-

cates that the amplification of the *erb*B2 gene in ovarian cancers leads to a bad prognosis for women with those tumors (also see *Science*, 12 May 1989, p. 654). "We think that the amplification plays a direct role in the pathogenesis of these tumors," Slamon says, "although we're not saying that it's the only alteration that may contribute."

Further support for the idea that the *erb*B2 gene is important in breast cancer comes from studies in which Vicki Chazin-Campbell of the Slamon group introduced extra copies of the human gene into cultured cells derived from human breast or ovarian cancers. Although those cells do not ordinarily form tumors when injected into nude mice, the ones with the extra *erb*B2 gene did.

Moreover, in a result that may have a direct application to therapy, the UCLA workers have shown that they can inhibit the growth of human breast and ovarian tumor cells that have been transplanted into nude mice by injecting the animals with a monoclonal antibody that binds to the *erb*B2 receptor protein. If the U.S. Food and Drug Administration gives the go-ahead, Slamon hopes to begin a clinical trial of the monoclonal antibody in patients with advanced breast cancer early next year.

When the *erb*B2 work began, incidentally, the growth factor supposed to work through the receptor was unknown. But Lippman and his Georgetown colleague Ruth Lupu have recently identified two growth stimulatory peptides that bind to the *erb*B2 protein. (Some of this work will be

Bombesin Receptor Gene Cloned

A late addition to the program at the Cold Spring Harbor cancer meeting generated a lot of interest among the assembled researchers on Sunday afternoon. The organizers invited Jim Battey of the National Institute of Neurological Disorders and Stroke to describe the cloning of the bombesin receptor, which has just been accomplished by his group and that of Eliot Spindel at the Oregon Primate Research Center in Beaverton.

Why the interest in the bombesin receptor? Well, Battey says, that's because "it does a lot of things in a lot of different places." The receptor takes its name from the peptide hormone bombesin, originally discovered in frogs as a modulator of muscle contraction. But it turned out that mammals have their own bombesin-like peptides, and they have a wide range of actions. They modulate nerve activity in the brain and other parts of the nervous system; they play a major role as stimulators of digestive secretions; and, more to the point for cancer researchers, the bombesin-like peptides stimulate the growth of several types of cells, including small-cell lung cancer (SCLC) cells, which makes it a target for cancer therapy (also see main story).

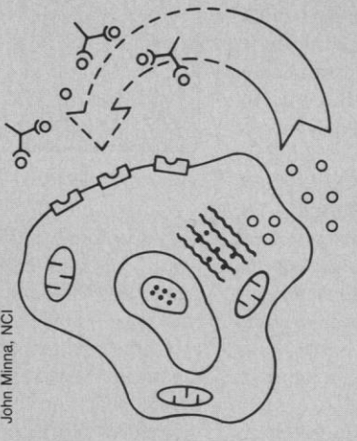
The SCLC connection was discovered several years ago by John Minna and his colleagues at the National Cancer Institute–Navy Medical Oncology Branch who found that SCLC cells both secrete and respond to a bombesin-like peptide. In addition, Frank Cuttitta of the Minna group found that an antibody he had made that reacts with gastrin-releasing peptide (the name for the mammalian bombesin made by the SCLC cells) inhibited the growth of the cells in culture and also blocked the growth of the SCLC tumors in nude mice.

That suggested that therapy directed at bombesin might be a way of treating SCLC, a malignancy that accounts for roughly 30,000 cases of lung cancer a year and is usually rapidly fatal. And, in fact, Minna and his colleagues have already begun a clinical trial to see whether treatment with the anti-bombesin antibody can help patients with the lung cancer. It's still too early to tell whether the therapy will benefit the patients, but it's at least encouraging, Minna notes, that they did not seem to experience toxic side effects.

The bombesin receptor is also a potential target for therapy—and perhaps a more specific one than the peptides themselves. Mammals have at least two different receptors, Battey points out, and their distribution is poorly understood. Do SCLC cells have both? Or just one? And would it be possible to design antibodies or drugs that work on an SCLC receptor, but not on those on nerve cells, for example?

Those are just a few of the questions that researchers would like to answer about bombesin receptors, but they have been stymied in their efforts because they weren't able to clone the receptor genes—until now. The cloning proved so difficult, Battey says, because human cells make very little of the receptor. That means that they contain few copies of the messenger RNA for the protein and that DNA libraries, which are made by transcribing the messengers into complementary DNAs, also contain few copies of the bombesin receptor DNA.

Consequently, both Battey and Spindel turned to Swiss 3T3 cells, a line of mouse fibroblasts that contain at least moderate numbers of the receptor—roughly ten times what the SCLC cells contain—for the source of the complementary DNAs that they would screen for the bombesin receptor gene clone. And both found such clones and have now compared the sequences, verifying that they have indeed found the same clone.



John Minna, NCI

Blocking a growth factor. *If growth factor molecules (small circles) released by a cancer cell bind to receptors on it, they stimulate its growth. That can be prevented by antibodies that bind either the growth factor or the receptor.*

The sequences also show that the protein encoded has the typical structure of a G protein–coupled receptor. That was expected: Biochemical studies had already shown that the bombesin receptor is a member of the large class of receptors that send their signals to the cell interior through G protein intermediates. But that is also an unusual mode of action for a growth factor receptor. Battey and Spindel say that as far as they know the bombesin receptor gene is the first gene for a growth factor receptor of that type to be cloned. The others analyzed to date have been tyrosine kinase that transmit their signals by adding phosphate groups to proteins inside the cell. Having the gene for the bombesin receptor should help clarify how this unusual receptor works.

It should also be possible to use the mouse bombesin receptor gene as a probe for pulling out the second mouse gene and for finding both human genes as well. And that should open the door to gaining a better understanding of how the bombesin-like growth factors contribute to the development of SCLC and perhaps to better therapies for this dangerous cancer. ■ **J.M.**

reported in next week's *Science*.) In keeping with the idea that activation of the receptor leads to aggressive tumor growth, the researchers find that the peptides are made just by cells from invasive breast tumors.

Several of the growth factors made by breast cancer cells, including the new ones identified by Lippman's group, act on the cells they came from. But others, including the fibroblast growth factors (FGFs), may act on other cell types.

The FGFs contribute to tumor growth by stimulating the formation of the blood vessels needed to nourish expanding tumors. They are therefore another potential target for cancer therapy. In fact, Lippman reported at the Cold Spring Harbor meeting that experiments done with Anton Wellstein, also at Georgetown, have shown that pentosan polysulfate, a carbohydrate related to the natural anticoagulant heparin, blocks the growth of mammary and other tumors in nude mice. The substance works, Lippman says, by binding to the FGFs.

Pentosan polysulfate is already used in Europe as an anti-anticoagulant and has so far shown little toxicity, Lippman says. In any event, the Georgetown group will be starting a clinical trial of the drug in patients with advanced breast and other types of cancers in the next week or two.

At least one other group has already begun a clinical trial of a therapy aimed at depriving a cancer of the growth stimulation it needs. Thomas Waldmann and his colleagues at the National Cancer Institute have chosen as their target the receptor for interleukin-2 (which is also known "T cell growth factor"). The gene encoding the interleukin-2 receptor has so far not been found to be a classical oncogene, but the rationale for the Waldmann group's therapy is essentially the same as that employed by the other researchers—use an antibody or other agent to stop a growth factor from stimulating cancer cell growth.

The interleukin-2 receptor is a good target for such therapy, Waldmann says, because it is present only on the surfaces of actively dividing cells, including those of several leukemias and lymphomas. Waldmann and his colleagues have treated 20 patients with one of those cancers, adult T cell leukemia, with a monoclonal antibody that recognizes and binds to one of the two proteins that together make up interleukin-2 receptor. Although the leukemia has proved refractory to other treatments, seven of the 20 who received the antibody have gone into remission, four of them complete.

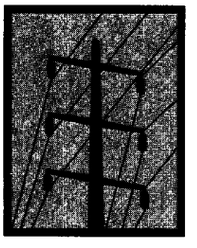
The treatment did not appear to cause toxic side effects, but there were other problems. Most of the remissions lasted only 5 to 8 months, Waldmann says, apparently because the patients eventually mounted an immune response to the therapeutic antibody, which was of mouse origin. And although the antibody could inhibit tumor cell growth by binding to the interleukin-2 receptor, it did not kill the cells. "It knew where to go, but didn't know what to do when it got there," as Waldmann puts it.

So now Waldmann and his colleagues are trying to develop new and improved antibodies that can overcome these problems. Cary Queen of Protein Design, Inc., in Palo Alto is "humanizing" the mouse monoclonal by substituting human antibody sequences for all portions of the molecule except those that bind the receptor. In addition, the NCI group is combining the antibody with toxins or radioactive isotopes that might be able to kill cells bearing the interleukin-2 receptor after the antibody binds.

The hope is that the oncogenes and the other growth regulators that have been so helpful to researchers in probing the biology of normal and malignant cells will also be reliable guides to cancer therapy. What will happen in the planned clinical trials remains to be seen. But if they should not prove successful, it's nice to known that there are many more genes that could also be potential targets for therapy. ■ JEAN MARX

Electromagnetic Fields: The Biological Evidence

Researchers now accept that even relatively weak EMFs have biological effects, but the evidence for health effects remains "iffy"



The second in a series.

OVER THE PAST FEW YEARS, epidemiological studies that seem to show links between exposure to electromagnetic fields (EMFs) and cancer—especially leukemias, lymphomas, and brain cancer—have generated headlines and prompted public concern about the hazards of living near power lines and operating electrical equipment. But while these studies are suggestive, they are sometimes contradictory and often lack statistical significance, and that has led most scientists to decide that the epidemiological data by themselves are inconclusive (see *Science*, 7 September, p. 1096). A recent draft report on EMFs and cancer, prepared by the Environmental Protection Agency, concludes, for example, that there is not enough evidence to classify the fields as "probable human carcinogens."

So researchers are studying how the body reacts to EMFs at the cellular level, in the hope that this will shed some light on the epidemiological findings. After more than a decade of laboratory experimentation, there is still no direct evidence that EMFs cause or promote cancer in lab animals. But during that time scientists have discovered a number of ways EMFs can affect biological functions, including changes in hormone levels, alterations in the binding of ions to cell membranes, and the modification of biochemical processes inside the cell, such as RNA transcription and protein synthesis.

Could any of these biological effects explain how EMFs might increase the risk of cancer? Some scientists think it's possible. Calcium ion concentrations in the cell, for instance, plays a major role in cell division, which in turn has an important part in cancer promotion. And recently, researchers at Battelle Pacific Northwest Laboratory in Richland, Washington, have come close to showing a direct EMF-cancer link in rats. They have found that EMFs suppress levels of the hormone melatonin, something that other researchers have shown makes female rats more susceptible to chemically induced breast tumors.

Despite these possible connections, "it's still not clear whether these biological effects translate into health effects," says Imre Gyuk, who manages the EMF research program at the Department of Energy. The Battelle work, for instance, hints at an EMF–breast cancer connection, but the epidemiological evidence pointing toward breast cancer is weaker than for leukemias, lymphomas, and brain cancers. Many of the laboratory experiments have been done at EMF intensities thousands of times higher than those people normally encounter at home or at work. And little of the data has been independently replicated by researchers in separate labs. As a result, Gyuk says, many of the results are still "iffy."

To some researchers, it is amazing that the