Research News

Many Gene Changes Found in Cancer

The common malignancies, including colon, lung, and breast cancer, apparently develop by a stepwise accumulation of mutations affecting both oncogenes and suppressor genes

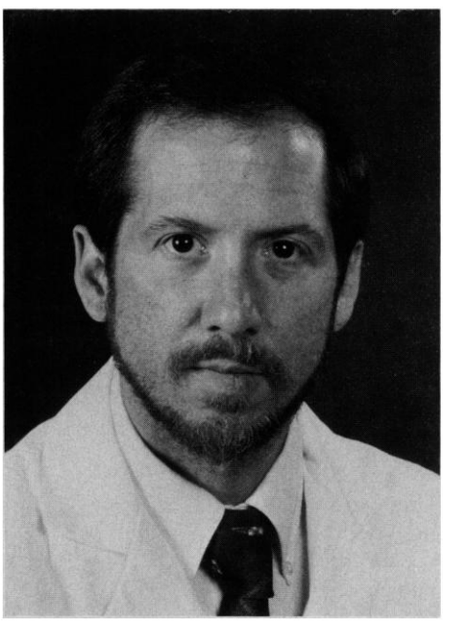
MAKING A CANCER CELL, it seems, is not all that easy. Researchers have found that as many as ten distinct mutations may have to accumulate in a cell before it becomes cancerous. "A few years ago the question was whether there are genetic changes in cancer cells," says veteran cancer cell biologist I. Bernard Weinstein of Columbia University's College of Physicians and Surgeons in New York. "Now you find a huge number of DNA changes. In cancer, the genome is shot to hell."

The researchers are finding that the changes affect the cellular oncogenes, activating them so that they can drive the increased cell growth and other abnormalities characteristic of cancer cells. But that is apparently not sufficient for the development of full malignancy. The changes also include the loss or inactivation of two, three, or even more "suppressor genes," which presumably work normally to inhibit cell growth.

The work on cancer cell mutations now provides compelling support for the idea that suppressor gene loss is at least as important in carcinogenesis as oncogene activation. A cell isn't driven to malignancy unless the brakes on cell growth are released at the same time the oncogenes are stepping on the accelerator.

The discovery of these extensive gene changes is not just of academic interest. The cancers in which they occur include the most common ones—the cancers of the lung, colon, and breast, which together account for about 40% of all human malignancies. The identification of the mutations underlying these cancers may aid efforts to prevent, diagnose, or treat the malignancies.

"If indeed you need 10 or 15 [gene] hits, and you could pick up people when they have only one or two, that would be pretty important clinically," says John Minna, who has been studying the gene changes in lung cancer at the National Cancer Institute–Navy Medical Oncology Branch and the Uniformed Services University of the Health Sciences in Bethesda, Maryland.



Tracking abnormalities. *Bert Vogelstein finds lethal cancers may have more mutations.*

Those individuals could then, for example, make sure that they avoid cigarette smoke or other agents that might produce the additional mutations that could propel their cells into malignancy. Or, if preventive efforts fail, close medical attention could help detect any cancers that do develop early on when they are most curable.

Early detection could make a significant impact on public health. For example, colon cancer can be cured surgically if the tumors are caught before they begin penetrating the colon wall. There are approximately 150,000 cases of colon and rectal cancer every year in the United States.

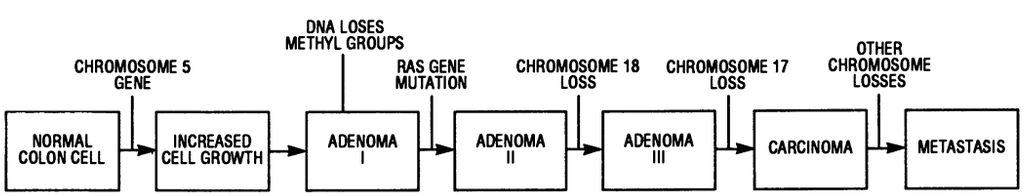
Analysis of the gene changes in tumor cells may also improve clinicians' ability to predict how their cancer patients will do, helping them identify those who need very aggressive therapy to control a highly malignant tumor. Researchers are already trying to do this by looking at changes in individual oncogenes, but these studies have sometimes led to conflicting results. Some investigators have found that a particular change indicates a poor prognosis, while others have failed to confirm such a link. Looking at several genes, instead of just one, may give more accurate predictions.

Support for this possibility comes from Bert Vogelstein of Johns Hopkins University School of Medicine and his colleagues. Earlier this year, they surveyed the gene abnormalities occurring in 56 colon cancers and then looked at the fates of the patients from whom the tumors had been removed. They found, Vogelstein says, "that cancers that have acquired more changes have a higher proclivity to kill patients."

In addition, researchers hope that understanding the gene alterations that cause cancer may help in the design of better treatments. If all the mutations they are seeing are in fact necessary for cancer to develop, then this may be a hopeful message from the therapy standpoint. "Multi-stage carcinogenesis gives more opportunities to intervene to stop or retard the process," says the NCI's Curtis Harris. "We should have multiple targets to direct our therapies toward."

So far, at least, the progression of gene changes leading to malignancy is best understood for colon and rectal cancers, largely as the result of work by Vogelstein and his colleagues. These cancers are especially well suited for the studies because they develop slowly, over years if not decades. The tumors begin as areas of increased cell proliferation in the colon lining, which can then progress to form the abnormal but noncancerous growths called adenomas, which finally become carcinomas when they acquire the ability to penetrate the colon wall.

The Johns Hopkins workers were able to



Long route to colon cancer. *Tumor suppressor genes are apparently lost from chromosomes 5, 18, and 17, and the* RAS *oncogene is activated, These changes usually occur in the order shown, but this order is not absolutely necessary.*

obtain surgical samples of the tumors in all of these stages and analyze their gene changes. They found the mutations accumulate gradually over time. "We think that it takes alterations in about five genes to get to the cancer stage," Vogelstein explains. "Small benign adenomas usually only have one recognizable gene change. As they grow in size they acquire three or four more."

The gene changes usually occur in a specific order, but there are exceptions. "It appears that it is the accumulation of changes, not the specific order, that is important for cancer development," Vogelstein says.

One of the genes altered is the *RAS* oncogene, which often undergoes an activating mutation about midway in colon cancer development. (*RAS* is so called because it was first identified in a virus that causes rat sarcomas.) But most of the other gene changes detected by Vogelstein and his colleagues are deletions of specific segments of certain chromosomes, principally num-

One Wilms' Tumor Gene Is Cloned; Are There More?

Cambridge, Massachusetts

A team of investigators has cloned a gene that may be the cause of Wilms' tumor, which accounts for 85% of all kidney cancers in children. The candidate gene, found on chromosome 11, has tumor-suppressing properties; it is only the third such suppressor gene to be identified. The recent cloning, however, does not fully resolve the question of how Wilms' tumor begins, because there is evidence that changes in several other genes (some not even on chromosome 11) may be involved in tumor initiation.

The isolation and characterization of the putative suppressor gene were reported at the recent annual meeting of the American Society of Human Genetics. The work was carried out by a team led by Katherine Call and her colleagues in David Housman's lab at the Massachusetts Institute of Technology, along with Carol Jones at the University of Colorado.

Wilms' tumor is most often diagnosed between the ages of 2 and 5, and the average risk for developing the tumor is about 1 in 8000. Although the prognosis is generally good, among a small subset of patients the death rate exceeds 50%.

The pair of genes seems to suppress tumor formation; when they are missing, a tumor may begin to form.

The Housman group based their identification on a comparison of chromosomes and expression profiles in normal kidney cells and cell lines derived from Wilms' tumor cells. In some tumor-cell lines, the newly identified gene was missing from both copies of the 11p13 region on the short arm of chromosome 11; little or no messenger RNA from the gene was detected in those cells. In normal cells the gene was present in two copies and was expressed. These findings suggest that in normal cells the pair of genes suppresses potential tumor formation; when they are missing, a tumor may begin to form. Since both copies must be altered for the gene to play a role in tumor formation, the Wilms' gene is recessive. The only other known recessive tumor-suppressing gene is the retinoblastoma gene, cloned in 1986. (The second known tumor suppressor gene, p53, seems to behave, at least in some cases, in a dominant fashion [see accompanying story]).

The differences between the retinoblastoma gene and the Wilms' tumor gene, however, may be more striking than their similarities. Only one gene has been implicated in the formation of retinoblastoma, whereas cytogenetic and molecular evidence suggests that the Wilms' gene is only one of several genes associated with that tumor. Grady Saunders and Louise Strong of the M. D. Anderson Cancer Center in Houston have found another sequence in the 11p13 region that may be implicated in Wilms' tumor; other groups have found abnormalities in other regions of chromosome 11 that may also play a role.

There is also evidence that genes on other chromosomes may be involved. Last year, two groups found that in some families with a predisposition to Wilms' tumor there was no apparent linkage to chromosome 11. Strong points out that a gene on another chromosome could initiate the sequence of events leading to Wilms' tumor by acting through a locus on chromosome 11. That other gene "may somehow predispose loss of a gene on 11p13 and/or may otherwise alter its expression," Strong says.

Even independent of its role in tumor formation, the putative Wilms' gene may turn out to be an important stretch of DNA. The partial sequence of the clone obtained by Housman's group shows that it contains a well-formed "zinc finger" region. This motif, named for its fingerlike projections, is thought to be a hallmark of transcription factors: proteins that bind to DNA and either enhance or suppress gene expression. And indeed the sequence already obtained does exhibit homology with other known transcription factors.

Whether the Wilms' gene is a transcription factor or not, its normal function seems to lie in the formation of the kidneys in the developing fetus. Evidence to that effect was presented last month at a meeting in Japan by Nicholas Hastie of the MRC Human Genetics Unit in Edinburgh. Hastie used Housman's gene to probe 7-week and 18-week human fetal tissues and he demonstrated that the gene was transcribed at both those times.

The finding that the Wilms' product is expressed in the developing kidney fits nicely with the histopathology of Wilms' tumors. The majority of these tumors contains a variety of cell types, which suggests, according to Bruce Beckwith of the Denver Children's Hospital, pathologist for the National Wilms' Tumor Study, that tumor induction probably begins during fetal development in a multipotential precursor cell. "Wilms' replicates both normal and abnormal kidney development," Beckwith says, and that is why it has "been referred to as a malignant embryonic kidney."

Beckwith joins other scientists in anticipating that the initiation of Wilms' tumor will not be limited to one genetic locus—or even a few. "It would surprise me," he says, "if only three loci were deranged. Kidney development is such a complex process that a lot of different things can go wrong." With regard to the recently cloned gene, he adds, the fundamental question is, "Does that gene lead to the precursor lesion, or does it activate the gene that does? We need to determine the gene's exact role, whether it's the immediate cause of malignant change or whether it acts prior to malignant change. As yet, either hypothesis could be valid." ■ MICHELLE HOFFMAN

Michelle Hoffman is a science writer based in Boston, Massachusetts.

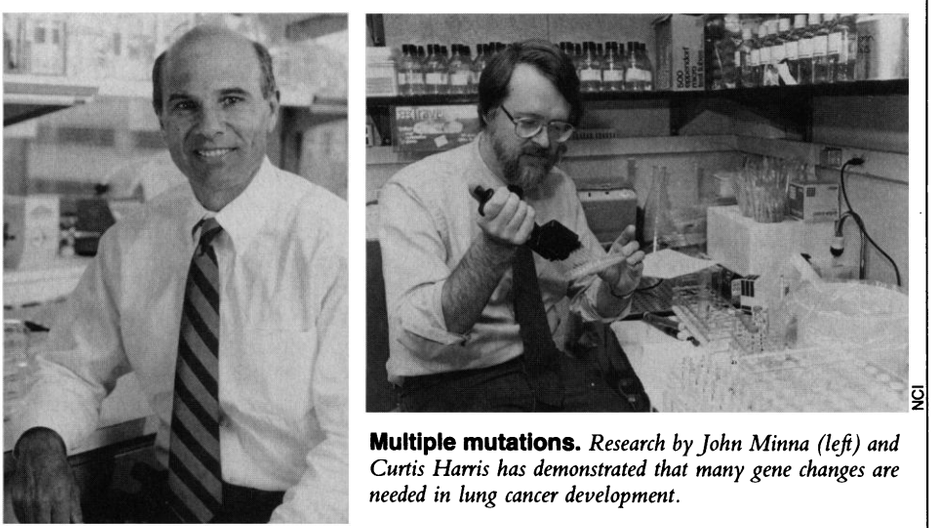
bers 5, 18, and 17. These most likely result in the loss of genes that could otherwise suppress tumor growth, Vogelstein says.

It usually takes two separate mutagenic events to get rid of a suppressor gene, because they occur in pairs and both may have to be inactivated in some way for their function to be lost entirely. That brings the minimum number of mutations that must accumulate to produce a colon cancer to eight to ten. And still more mutations may be required to give cancer cells the ability to spread to distant sites in the body.

So far, the Johns Hopkins workers have identified only one of their suspected suppressor genes. They have found that the deleted segment of chromosome 17 carries a suppressor gene; its loss contributes to the development of retinoblastomas, an eye tumor of children.

Deletions of chromosomes 3 and 11 also occur frequently in lung cancers, but not in colon cancers. And conversely the chromosome 5 and 18 deletions are found frequently in colon cancers, but not in lung cancers.

At least one thing is clear from these findings. The fact that so many mutations are needed for cancer development shows that cells normally control their growth very closely with multiple sets of checks and balances. "From an evolutionary standpoint that is very important," Harris says. "You don't want to go from a normal cell to a cancer cell in one step."



NCI

Multiple mutations. *Research by John Minna (left) and Curtis Harris has demonstrated that many gene changes are needed in lung cancer development.*

gene designated p53 that encodes a protein with known tumor-suppressing activity. (The gene is designated p53 because its protein product has a molecular weight of 53,000.)

Loss or inactivation of the p53 gene may in fact contribute to the development of several common cancers. Minna and his colleagues, and also Vogelstein's group in collaboration with that of Harris at the NCI, have found that the gene is frequently missing or abnormal in lung cancer cells. And the same is often true for breast cancer cells as well, according to the NCI's Robert Callahan.

But while some gene changes may be common to the different cancers, others are not. For example, Minna, Frederick Kaye, and their NCI colleagues find that the retinoblastoma gene is missing or defective in nearly all small-cell carcinomas of the lung (SCLCs), a malignancy that accounts for about one-third of lung cancer cases. The gene is also frequently deleted in other forms of lung cancer, according to Harris. But it does not appear to be affected in colon cancers. The retinoblastoma gene is another

The findings are also consistent with the epidemiology of colon, lung, and breast cancer, all of which occur most commonly in middle age or later. It takes time to accumulate the necessary burden of mutations. This may mark a point of difference between these cancers and those that develop early in life. Fewer gene changes may be required to give pediatric tumors such as retinoblastoma and the lymphomas and leukemias that afflict mainly younger people.

At present, however, it is still safe to say that researchers have more questions than answers about what all the gene changes seen in cancer cells mean to either normal or cancer cell biology. They are still in a cataloguing stage and what they are finding is very complex.

Understanding what is happening is "going to be like putting a jigsaw puzzle together," says Sandra Bigner of Duke University Medical Center. Bigner has been working with Vogelstein in analyzing the gene changes that occur in glioblastomas and medulloblastomas, respectively the most common brain tumors of adults and children. These cancers also show several gene changes, affecting both oncogenes and suppressor genes.

Researchers would like to know, for example, what causes all of the mutations. Some may be inherited, as is often the case for the retinoblastoma gene abnormalities. Others may be caused by environmental carcinogens. Harris has noted that gene deletions occur more frequently in SCLC and squamous cell carcinoma of the lung, two cancers that are closely associated with cigarette smoking, than in adenocarcinoma of the lung, in which smoking appears to have less of a causative role.

And cells that are undergoing malignant transformation are inherently prone to mutate. This may be partly because they are growing abnormally fast, thereby increasing the opportunities for mutations to occur. Vogelstein's group has also noted that early in colon cancer development, the cell DNA shows a loss of methyl groups. He does not know what causes this loss, but suggests that it may cause the chromosomes to adhere to one another at a time in mitosis when they should instead be moving apart into the forming daughter cells. The decreased DNA methylation may therefore contribute to the chromosomal abnormalities of cancer cells.

The researchers also want to know what the proteins encoded by the genes that are mutated during carcinogenesis do. This information is very important as a guide to normal cell growth control and to potential therapeutic strategies. And it is by and large not yet available. Only two putative suppressor genes—the retinoblastoma gene on chromosome 13 and the p53 gene on chromosome 17—have been identified so far, although other suppressor gene candidates have been found, including one for a Wilms' tumor gene on chromosome 11, which is one of those frequently found to carry deletions in lung and other cancers (see p. 1387).

There are at least some clues about what the retinoblastoma and p53 proteins do. Both are located in the nucleus, where they may act to turn genes on or off. Those target genes have not yet been identified, however. They are among the many pieces still missing from the jigsaw puzzle. ■ **JEAN MARX**

ADDITIONAL READING

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