Learning How to Suppress Cancer

Fast and furiously, researchers are identifying new "tumor suppressor genes," which promise a new understanding of cancer—and perhaps a clinical payoff

For most of the nearly 20 years that researchers have been studying the genetic changes that might cause cancer, the "oncogenes" have dominated the scene. They've garnered so much attention because these genes, which normally regulate cell growth and development, have the potential to malfunction in a dangerous way. When they are altered, their abnormal activity can cause cells to grow out of control, mimicking at least one major feature of cancer. Within the past few years, however, it's become increasingly apparent that oncogene mutations aren't the only—and perhaps not even the most important—genetic changes contributing to the development of human cancers.

Among the other changes researchers are now finding play a key role in the development of human tumors are the alterations that inactivate "tumor suppressor genes." These suppressors apparently act to keep cell growth in check—thus providing the flip side of the oncogene story—and their inactivation has been linked to the development of a wide variety of human cancers, including breast, colon, and lung cancer. Because of their obvious scientific and clinical relevance, these normally protective genes have aroused soaring interest among cancer researchers.

Take just one indicator of that interest: the rapid growth of papers featuring the *p53* tumor suppressor. There's a good reason why researchers would be interested in *p53*. After all, changes in this tumor suppressor may help cause as many as 50% of *all* human cancers. But even given the gene's central role, the increase in the number of *p53* papers is startling. Data compiled by Philadelphia's Institute for Scientific Information show that the number of titles mentioning *p53* has been doubling every year since 1989, the year its tumor suppressing capabilities were first recognized (see graph on p. 1386).

But, for all its fascination for cancer researchers, *p53* is hardly the only center of interest in the field of tumor suppression. In fact, the number of suppressor genes is growing right along with researchers' interest. In the past 6 months alone, at least three new tumor suppressor candidates have been discovered, bringing the total number to about 10—and significantly enlarging the range of known activities of the proteins encoded by these helpful genes.

New optimism

As in the case of the oncogene products, the tumor suppressor proteins are now found throughout the cell, from the outer membrane to the nucleus in the interior. And while there is much to be learned about what the proteins encoded by the suppressor genes do, researchers are making progress toward understanding how these proteins normally function—and also how they may malfunction in cancer. "I'm so optimistic now. Not only are the genes being found, but we're getting clues to how they function," says Curt Harris of the National Cancer Institute (NCI), whose own research deals with gene changes in lung cancer.

Those clues to function could point to improved diagnosis—even treatment—of cancer. For example, animal experiments suggest that it may be possible to quash the tumor-forming capacities of cancerous cells by using genetic engineering to insert a normal copy of a tumor suppressor gene into them. Alternatively, it might be possible to find therapeutic drugs that mimic the suppressor's normal function. While the therapeutic strategies may not come to fruition soon, researchers are already testing the diagnostic and prognostic possibilities of the altered suppressor genes. Says molecular biologist Arnold Levine of Princeton University, a *p53* expert: "For the first time we are getting the ability to translate between the molecular biology [of tumor suppressor genes] and clinical application."

While researchers' interest in tumor suppressors is soaring now, it wasn't always that way. Indeed, the field got off to a slow start. Most tumor suppressor researchers credit experiments done in the late 1960s by molecular biologist Henry Harris of Oxford University and his colleagues with providing the first evidence for tumor suppression. When the Oxford workers fused normal rodent cells with cancer cells, they found that some of the resulting hybrids lost their ability to form tumors. Although this result suggested that the normal cells were supplying some tumor-suppressive activity, that conclusion did not meet with widespread acceptance, partly because the results were not absolutely consistent: Some of the hybrids did not lose their tumorigenic properties.

That wasn't the only problem in the early days of the suppressor research. Another was that the tumor suppressor pioneers lacked something the oncogene researchers had from early on—a good assay for finding their genes. Oncogenes can be readily detected in the laboratory because they stimulate the growth of cells in culture. But as longtime tumor suppressor researcher Eric Stanbridge of the University of California, Irvine, points out, putting a suppressor gene into cultured cells either kills them or causes no discernible

SOME KNOWN OR CANDIDATE TUMOR SUPPRESSOR GENES

Gene	Cancer Types	Product Location	Mode of Action	Hereditary Syndrome
APC	Colon Carcinoma	Cytoplasm?	?	Familial adenomatous polyposis
DCC	Colon Carcinoma	Membrane	Cell adhesion molecule	——
NF1	Neurofibromas	Cytoplasm	GTPase-activator	Neurofibromatosis type 1
NF2	Schwannomas and meningiomas	Inner membrane?	Links membrane to cytoskeleton?	Neurofibromatosis type 2
p53	Colon cancer; many others	Nucleus	Transcription factor	Li-Fraumeni syndrome
Rb	Retinoblastoma	Nucleus	Transcription factor	Retinoblastoma
RET	Thyroid carcinoma; pheochromocytoma	Membrane	Receptor tyrosine kinase	Multiple endocrine neoplasia type 2
VHL	Kidney carcinoma	Membrane?	?	von Hippel-Lindau disease
WT-1	Nephroblastoma	Nucleus	Transcription factor	Wilms tumor

changes, although the cells may no longer be tumorigenic in animals. So researchers looking for suppressor genes were forced to look for other methods. But those methods weren't quick in coming, and the result was that the work didn't take off until the 1980s, when the invention of "positional cloning" methods made suppressor gene hunts possible.

In this approach, researchers first look for genetic variations indicating the presence of mutant genes in cancer cells or in the cells of patients with inherited cancer susceptibilities. Then they gradually zero in on the mutated sites until they get their genes. This method can be tedious, but it paid off for the researchers, because they were looking directly at the gene changes associated with human cancers. That contrasts with the situation in oncogene research, where the oncogenes were generally identified by their growth stimulatory effects on mouse cells or by their sequence similarities to known genes. And while those studies are shedding considerable light on growth-control pathways, relatively few of the oncogenes identified this way have turned out to have much of a role in human tumors.

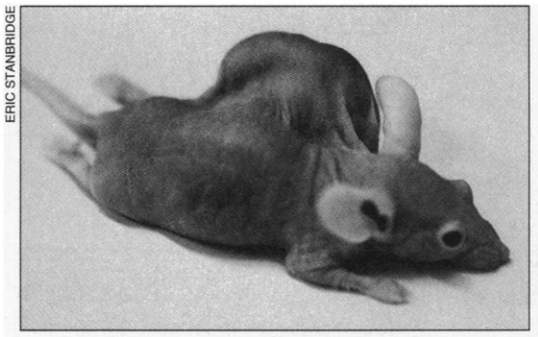
The first tumor suppressor gene to be identified was the retinoblastoma (*Rb*) gene, which causes a hereditary eye tumor of children. Although its cloning in the mid-1980s by three groups was not quite positional cloning, many of the same DNA-analyzing techniques came into play. (One of the three groups included Stephen Friend, who was then a postdoc in Ted Dryja's lab at the Massachusetts Eye and Ear Infirmary, and Robert Weinberg of the Massachusetts Institute of Technology; the other two were led by Wen-Hwa Lee of the University of California, San Diego, and William Benedict of the Center for Biotechnology in Woodlands, Texas.) Since then, however, positional cloning has been used to identify genes for several additional human hereditary cancer susceptibilities (see table).

The development of positional cloning techniques was only one reason why the field of tumor suppressors has taken off in the past few years. Another was the identification of the *p53* gene as a suppressor, combined with the recognition that mutations in the gene are found in so many tumors. Ironically, given the opposed roles of the two types of genes, *p53* didn't start its life as a suppressor; for 10 years after its discovery in 1979 it was thought to be an oncogene. The main reason for the mischaracterization, it turned out, was that researchers were working with a mutant version.

But in 1989, work by several groups, including Levine's and that of Bert Vogelstein at Johns Hopkins University School of Medicine, revealed that the nonmutant, wild-type *p53* is actually a tumor suppressor. Apparently, many of the mutations in tumor cells that rob the *p53* protein of its tumor-suppressing power also make it behave like an oncogene. And that, as Levine points out, may make *p53* mutants doubly dangerous and help explain why they occur in so many cancers. A graphic demonstration of that came about 3 years ago, when a team led by Stephen Friend, whose lab is now at Massachusetts General Hospital in Boston, found that *p53* is the gene at fault in Li-Fraumeni syndrome, a very rare hereditary cancer susceptibility that predisposes to breast cancer and several other types of tumors as well.

Picking up speed

The combination of the advances achieved by positional cloning and the identification of *p53* as a tumor suppressor sent the field speeding on its way. Another reason for the heightened interest is the finding that even those tumor suppressors that were originally identified as the cause of rare inherited cancer susceptibilities are also turning out to be implicated in the much more common, "sporadic" cancers, in which inheritance does not seem to be a factor. While none as yet matches *p53*'s frequency in many types of human tumors, researchers find that a mutation that confers a rare hereditary susceptibility to colon cancer, say, or kidney cancer, also commonly occurs in the corresponding sporadic tumors. "Looking at these rare diseases is leading to a better understanding of cancer in general," says NCI's Harris.



Subduing a tumor. Human colon cancer cells injected on the left side of the animal produced a large tumor. But the same type of cells, carrying a newly introduced chromosome with a tumor suppressor gene, produced no visible tumor on the right.

In order to realize the full potential of tumor suppressor genes for understanding of cancer in general, researchers want to move beyond implicating individual tumor suppressors in specific types of cancer and get a handle on just where—and how—the tumor suppressors operate within the cell.

Again, *p53* is one of the leaders. Researchers are now beginning to get a handle on what the gene does to protect the cell from becoming cancerous. Work from several labs suggests, for example, that the normal *p53* protein serves to protect the genome against DNA-damaging agents. It apparently has the capacity to sense when damage occurs and then halt cell division until the damage is repaired, or, if the damage is too severe to be corrected, triggers apoptosis—programmed cell death—to get rid of the damaged cells entirely. But when the gene is mutated, the cells may keep on dividing, allowing the DNA damage to build up. That's a dangerous development because it might lead to further mutations that would increase cancer cells' malignant potential or make them resistant to chemotherapeutic drugs. Researchers have also found that the *p53* protein's tumor suppressive effects may depend on its activity as a factor that helps to regulate gene transcription, the first step in gene activity.

P53 isn't the only tumor suppressor gene that encodes a transcription factor. Indeed, all of the first three tumor suppressors identified—*p53*, *Rb*, and *WT-1* (a susceptibility gene for a childhood kidney cancer called Wilms tumor)—produce transcription factor proteins. For all three genes, researchers suppose their tumor suppressing effects depend on their activity as transcription factors. With one or two exceptions, however, the specific target genes they regulate remain to be discovered.

The burst of recent work, however, is showing that tumor suppressor activity is not limited to the cell's nucleus and the DNA contained there. "The localization of the gene products and their functions are quite varied," says Vogelstein. His group has evidence, for example, that a suppressor gene called *DCC* (for deleted in colon cancer) may be as far from the nucleus as it's possible to be: at the cell's outer membrane, where it could be involved in cell-cell adhesion.

The protein encoded by the neurofibromatosis type 2 (*NF-2*) gene, another of the tumor susceptibility genes cloned this year, may also be located in the membrane, or perhaps just under it. Yet this protein is thought to have a function quite different from controlling cellular adhesion. "*NF-2* is a member of a family of proteins typically viewed as connecting the membrane to the cytoskeleton," says Jim Gusella, the leader of one of the groups that cloned the gene. (The other was led by Gilles Thomas of Institute Curie in Paris.) The cytoskeleton is a network of protein filaments extending through the cell whose functions include helping the cell maintain its shape. This complex structure often becomes disorganized in cancer cells. This has generally been thought to be a secondary effect of the loss of growth control, Gusella notes, but the *NF-2* work suggests that the opposite may also be true: Cytoskeletal disorganization may lead to abnormal cell growth.

Another tumor suppressor gene that

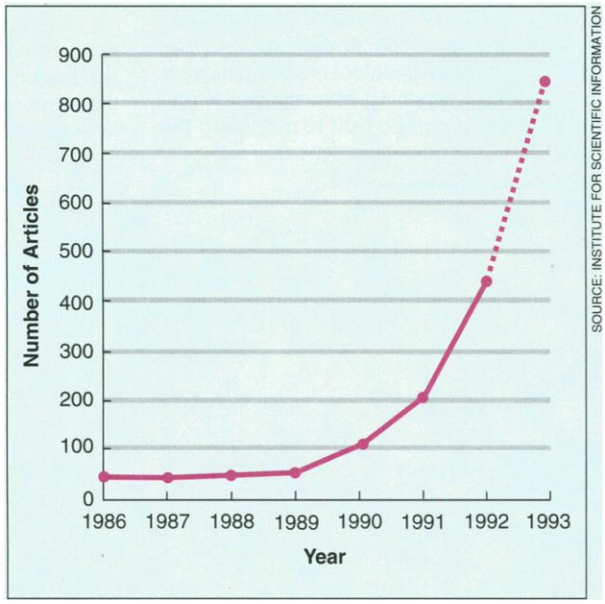
might be located at or near the membrane was cloned by Michael Lerman and Bert Zbar of NCI and their colleagues this year. This gene also causes a hereditary cancer susceptibility, in this case, a condition known as von Hippel-Lindau disease, in which patients develop kidney carcinomas. The location of the protein encoded by the von Hippel-Lindau gene is not known but there are hints in its structure that it, too, might be a membrane protein.

As knowledge of the tumor suppressors has grown, it has become clear that they do not reside only in the cell's nucleus or near its membrane. Other tumor suppressor products end up in the zone between the nucleus and outer membrane: the cytoplasm. The first of these to be discovered is the neurofibromatosis type 1 (*NF-1*) gene, which was cloned independently in 1990 by Francis Collins' group at the University of Michigan and Ray White's at the University of Utah. *NF-1* primarily causes benign tumors of the peripheral nerves. The protein encoded by the gene turns out to operate in the cytoplasm and to be an activator of an activity of the *ras* oncogene proteins. Specifically, *NF-1* stimulates *Ras* to split the energy-containing molecule GTP. Since *Ras* proteins are inactivated when GTP is split to GDP, *NF-1* may act to keep this oncogene in check.

Identifying the function of the *NF-1* protein not only adds to the repertoire of activities and sites occupied by the tumor suppressor genes. It also shows that tumor suppressor and oncogene products interact with each other as well as with members of their own class. Further evidence along those lines was provided by Levine's group, which found that the *p53* protein interacts with the protein product of an oncogene called *mdm2*. This may help explain *mdm2*'s oncogenic action, since by binding to *p53*, it blocks the tumor suppressor's effects.

In view of the fact that Levine's group also finds that *p53* itself stimulates production of *mdm2*, the two proteins appear to participate in a feedback loop that helps keep *p53* activity and *mdm2* synthesis in the correct balance for normal cell growth. Disturbing either one might therefore help throw the cell into the excess growth of cancer. In fact, Levine, with Carlos Cordon-Cardo of Memorial Sloan-Kettering Cancer Center, has been looking at the two genes to see if they might be of value as prognostic indicators for cancer patients—and preliminary results suggest they are. Patients tend to do poorly if their tumors have a *p53* mutation, Levine says, and even worse if they have both a *p53* mutation and amplification of the *mdm2* gene.

And *mdm2* isn't the *p53* protein's only partner. Earlier this year, Frank Rauscher of the Wistar Institute in Philadelphia and Daniel Haber of Massachusetts General Hospital and their colleagues found that the *p53* protein interacts with the protein encoded by *WT-1*. "The fact that all these players interact with one another means there's a regulatory network," says Levine, although researchers are still a long way from knowing exactly how it functions.



Suppression explosion. The number of papers on the *p53* tumor suppressor gene has been doubling annually since 1989. (The 1993 total is projected from 429 papers by 30 June.)

Clinical applications

Even with much to learn about how the products of tumor suppressors work and how they interact with the products of oncogenes, the tumor suppressor research is already beginning to make its way into clinical application. Since mutations in so many of the genes have been fingered as the culprits in hereditary cancer susceptibilities, at the very least, identifying a range of tumor suppressors should make it easier to identify members of families who carry the mutant genes and are thus at high risk of developing cancer.

In addition, several groups besides that of Levine and Cordon-Cardo have evidence that finding certain types of mutant *p53* genes in tumors may signal a poor prognosis for the patients. In the 16 June issue of the *Journal of the National Cancer Institute*, for example, a group led by Umberto Veronesi of the National Institute for the Study and Cure of Tumors in Milan, Italy, reported that increased expression of a mutant *p53* protein correlates with faster relapse and decreased survival in women with node-negative breast cancer.

Diagnostic information is, of course, desirable, but preliminary results also suggest that *p53* and other tumor suppressors may make good therapeutic targets. One study comes from Stanbridge's group, which introduced chromosomes carrying one of three different tumor suppressor genes (*p53*, *APC*, or *DCC*) into human colon cancer cells. Doing so, they found, completely suppresses the cells' capacity to cause tumors in nude mice (used because their defective immune systems allows foreign tissue to grow). And that result was achieved even though several gene defects are required to make colon cells cancerous. "You can have a cell line with multiple defects and correction of any one [tumor suppressor defect] reverses the malignant state. That's obviously good news for therapeutic application," Stanbridge says.

One strategy for making the most of that good news is to find a way to genetically engineer a good copy of a tumor suppressor gene into cancer cells. Another may be to find drugs that can restore the tumor suppressive effects of a defective protein.

A suggestion that the latter strategy could pay off comes from David Lane and colleagues at the University of Dundee, Scotland. Lane's group has been studying regulation of the *p53* protein's activity. Certain mutant versions of *p53* found in tumor cells apparently lose their responsiveness to the cellular signals that would normally turn them on. But Lane and his colleagues have found that, in fact, the mutants aren't completely unresponsive. They can be activated artificially by an antibody that binds to the proteins. "We're trying to study this regulation in detail to find therapeutic agents that can bring dead *p53* back to life," Lane says.

Whether dead *p53* or other tumor suppressor genes can be resurrected in human tumors remains to be seen, of course. Another question that remains to be answered is how many tumor suppressor genes there are. While the researchers who are working on tumor suppressors have a long way to go to match the total number of oncogenes so far identified—close to 100—it is likely that other tumor suppressors are out there in the genome, waiting to be discovered. Indeed, it's clear that the genes for all hereditary cancer susceptibilities, most of which have turned out to be tumor suppressors, haven't yet been found. One particularly hot candidate at the moment is the breast cancer susceptibility gene, the target of an intense search (*Science*, 29 January, p. 622). Whether that particular tumor suppressor is found soon or not, it's clear that the excitement surrounding this field is not going away soon.

–Jean Marx