FEATURE

Fingering Carcinogens with Genetic Evidence

MARGIE PATLAK

Predictable mutation patterns in DNA give researchers another tool to identify potential environmental carcinogens.

Recent studies suggest that some cancer-causing agents leave distinctive genetic fingerprints in the tumors they trigger. Exposure to a mold toxin, for example, causes a highly specific pattern of mutations in liver tumors. These studies are inspiring researchers to use the fingerprints to pinpoint environmental causes of cancers, rule out some suspected cancer agents, and discover causes for cancers that were missed in traditional epidemiology studies that tried to link exposure to certain chemicals with the risk of developing cancer.

Regulators such as EPA are eyeing this new avenue of research with enthusiasm because of its potential usefulness in human risk assessment. "We're customers ready to receive these kinds of results," said Jeanette Wiltse, chair of the technical panel that wrote EPA's new cancer risk assessment guidelines. She pointed out that human studies aimed at linking an increase in cancer in a specific population to exposure to certain environmental contaminants often have findings that can be interpreted in several different ways. "If there's the potential for three things to cause a positive result in a study and we have this kind of information to tell us which one it is, then we'll do a much better job at pinpointing what the problem is," she said.

EPA's new cancer risk assessment guidelines specify that its regulatory decisions be based more on findings such as genetic fingerprints that suggest a carcinogen's mechanism of action and on a broad range of evidence, including genetic data, than on whether a compound causes tumors in animals.

The genetic code and cancer

Advances in genetic fingerprinting build on the understanding of the genetic root of cancer that has emerged in recent decades. Buried in the nucleus of cells are strands of DNA made of bases—adenine, thymine, guanine, and cytosine—in a particular order that spells out the instructions for the production of thousands of proteins. Each triplet of bases, or codon, along the DNA strand codes for an amino acid or for a signal to start or stop building amino acid chains, which comprise proteins. If genetic damage changes base order through additions, substitutions, or deletions, the cell may produce either a faulty protein or the right protein in the wrong amounts—mistakes that can result in cancer.

Cancer is thought to result from the sequential or simultaneous genetic damage to several genes, such as *ras* and *p53*, which

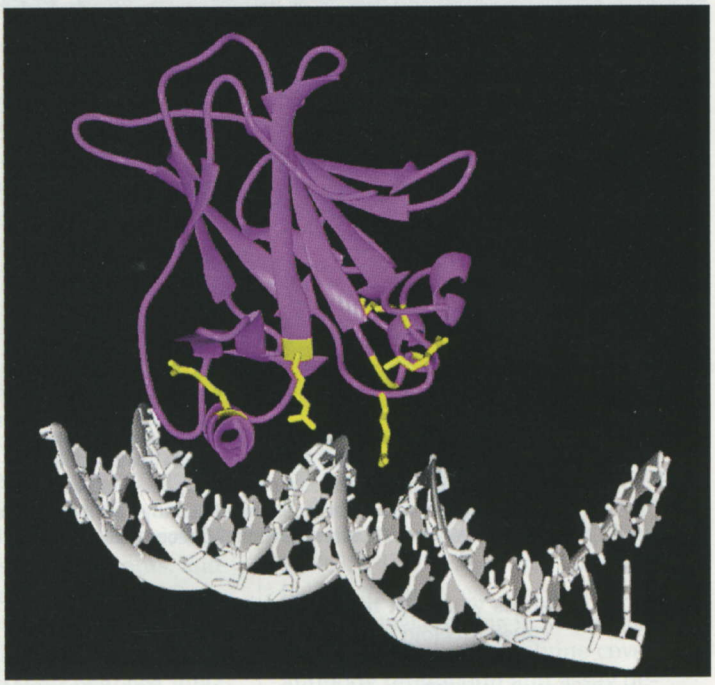
0013-936X/97/0931-190A$14.00/0

govern cell growth, death, and maturation. Known as the guardian of a cell's genes, *p53* prevents cells from dividing if any of their DNA has been significantly damaged. If the DNA can't be repaired, *p53* triggers cells to commit suicide. Because of this beneficial role, *p53* is known as a tumor suppressor. When *p53* is damaged, however, this Dr. Jekyll can turn into a Mr. Hyde and foster the growth of cancers not only by not preventing DNA-damaged cells from dividing, but also by fostering the production of proteins that encourage the growth of tumors. This double-edged sword may explain why *p53* mutations are found in more than half of all cancers (*1*).

Although many people inherit genes that make them susceptible to developing cancer, mutations in tumor-causing or tumor-suppressing genes are rarely inherited from parents. (Some studies, in fact, have suggested that seriously flawed genes inherited from parents probably explain no more than 5% of all cancers in the United States.) Mutations usually are triggered by external environmental agents, such as cigarette smoke or radon. Compounds the body produces internally while breaking down food or carrying out other vital tasks can also generate cancer-causing mutations. Mutations can also arise if a cell miscopies its own DNA and doesn't use its repair mechanisms to fix the errors before dividing.

The pattern of some of these mutations is incriminating. In 1991, Douglas Brash of Yale University and his colleagues showed that ultraviolet radiation causes adjacent cytosine bases in the *p53* gene to be replaced with thymines. The researchers have found such mutations in nearly all of one type of nonmelanoma skin cancers they studied and in 50% of the other type. About the same time, Curtis Harris of the National Cancer Institute and his colleagues found that high levels of exposure to aflatoxin—a mold toxin that contaminates grains in economically depressed countries—were linked to mutations in the third base of a specific codon of *p53*, called 249, in most of the liver cancers they examined.

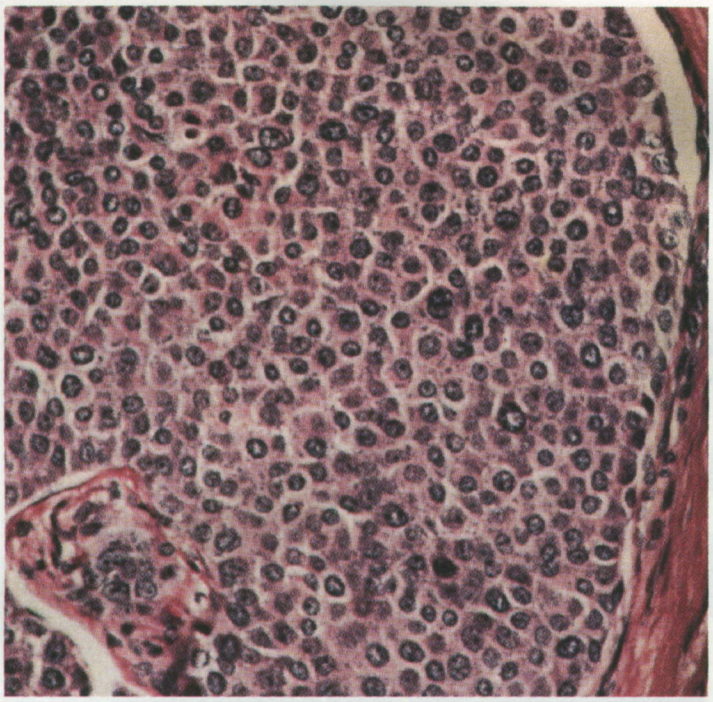
In 1994, Monica Hollstein and colleagues at the German Cancer Research Center found that all the tumors of a rare type of cancer, called angiosarcoma, that were removed from plastics factory workers exposed to high levels of vinyl chloride had numerous adenines scattered throughout *p53* that were replaced with thymines. Three years ago, when Jack Taylor of the National Institute of Environmental Health Sciences examined the lung cancer patterns of uranium miners exposed to high levels of radon, he found that one-third of them had mutations in the second base of the 249 codon of *p53*, whereas most lung cancers isolated from cigarette smokers not exposed to high levels of radon usually lack this specific mutation but have other distinctive patterns of mutations (*1*, *2*).



Mutations in the gene known as *p53* (depicted in purple) can lead to a variety of cancers. By studying groups of people to find particular patterns of anomalies along specific portions of this gene, scientists can sometimes determine whether an environmental contaminant triggered the mutations. (Model was produced by Tom Darden and Bill Beard, National Institute of Environmental Health Sciences, using data from the Brookhaven Protein Data Bank)

The mutation spectra can also indicate which compound in a mixture suspected of being carcinogenic is the most likely cancer-causing agent. There are several carcinogens in cigarette smoke, for example, including 4-aminobiphenyl and oxygen radicals. The former was thought to be the cause of bladder cancers in people who smoke cigarettes, because studies had shown it bound to the DNA of human bladder cells. A 1993 study done by Peter Jones of the University of Southern California, however, found that the types of mutations seen in the bladder tumors of smokers are more typical of what is induced by oxygen radicals, not 4-aminobiphenyl. These findings suggest that oxygen radicals are the most likely trigger of bladder cancer in people who smoke (*3*).

Researchers analyzing the mutations seen in acute myeloid leukemia are detecting likely environmental causes of the cancer that standard epidemiology studies have missed. These studies found an only marginally increased risk of the disease in people with occupational exposures to certain chemicals, including gasoline and compounds used to manufacture paper, furniture, textiles, or paint. But Taylor found that in people with a specific *ras* mutation, the risk of developing the leukemia was strongly tied to exposure to these chemicals. He suggested that such expo-



Pinpointing the environmental triggers that cause breast tissue to become cancerous (cells above) has been difficult. Because *p53* mutations in breast cancer vary geographically, scientists hope these differences might identify carcinogens in specific regions. (Courtesy National Cancer Institute)

sures were not implicated in previous studies because myeloid leukemia consists of several subtypes, each probably caused by a different agent. Exposure to no specific agent, consequently, could be shown to boost the risk of developing all types of the leukemia. Genetic fingerprinting allowed him to pinpoint a subtype of myeloid leukemia that was linked to exposure to chemicals used in certain occupations (*4*).

Sorting out breast cancer sources

Investigators are using telltale mutations to help make sense of the perplexing findings on breast cancer that have accumulated over the past decade. A small proportion of women may inherit genes that make them particularly susceptible to breast cancer. Others have a hormonal predisposition for developing the malignancy. But most researchers believe environmental triggers are key to the majority of cases. Several findings support this assumption, including the finding that women who migrate to new regions of the world adopt the breast cancer risk of those nations and not that of their country of origin.

But several studies aimed at discovering environmental causes of breast cancer have produced inconsistent or inconclusive findings. The inconsistencies might stem from the improbability that an environmental exposure implicated in a study done in one region of the world would be confirmed by similar studies done in other regions of the world where different environmental triggers of breast cancers may be more common.

In support of this hypothesis, Steve Sommer and John Kovach of the City of Hope National Medical Center in Duarte, Calif., found that the *p53* mutation patterns in breast cancers of women from various parts of the world differed dramatically. However, this wasn't true for the lung cancer *p53* mutation patterns, which were the same for most smokers—even those living in different parts of the world. Excluding breast cancer, no other cancers have been shown to have such a wide repertoire of *p53* mutation patterns (*5*).

"Our findings suggest that the environmental contribution to breast cancer may be more complex than most, if not all, cancers," said Sommer. To find the offending agents in breast cancer, he suggested using animal models to screen likely environmental carcinogens within a specific region to see if they cause the same pattern of *p53* mutations seen in the breast cancers of women in that region. A match would offer some evidence that the specific compound was a cause of breast cancer in the region, although additional epidemiology studies would have to be done to verify this deduction. "This approach gives you clues as to what mutagens in the environment are important in causing the cancer," Sommer said.

But many scientists question the validity of using solely mutation patterns to incriminate environmental compounds as triggers of cancer. Although in a few cases the mutational fingerprint is unique to a specific agent, in other cases the mutation patterns for different chemicals overlap. Both oxygen radicals and ultraviolet light can cause adjacent cytosine bases in the *p53* gene to be preempted by thymines, for example. As more research is done, more overlap may be found.

"I doubt that there will ever be a unique fingerprint mutation associated with a particular carcinogen that can't be caused by another one," said Taylor. He added that such overlap doesn't negate the usefulness of genetic fingerprints to "help us identify which compounds are likely to be cancer-causing agents and raise our level of suspicion that certain exposures are truly causes of a cancer, but I don't think anybody is willing to take a legal stand about this."

Most researchers concurred that mutation pattern findings can add to the weight of evidence that a particular compound causes a particular cancer, but the findings can't stand on their own. "In any one tumor, the mutation seen is unlikely to be distinctive. After all, for a given base, there are only five ways it can go wrong: change to one of the other three, deletion, or duplication," says Brash. "Thus, it would seem that the only conclusions that would hold up in court would be those involving many individuals, as in a class action suit."

These limitations don't seem to be dampening the enthusiasm of many researchers. "The results that are emerging from this area of research are going to be highly relevant to regulation," said Sommer. "We have these powerful tools, the paradigm has been worked out, and now it's up to labs to apply it." Added Wiltse, "The only thing that hampers the usefulness of mutation patterns research is that not enough people are doing it yet."

References

(1) Greenblatt, M. S. et al. *Cancer Res.* **1994**, *54*, 4855–78.
(2) Hollstein, M. et al. *Carcinogenesis* **1994**, *15*, 1–3.
(3) Spruck, C. H. et al. *Cancer Res.* **1993**, *53*, 1162–66.
(4) Taylor, J. et al. *The Lancet* **1994**, *343*, 86–87.
(5) Hartmann, A. et al. *Trends in Genetics* **1997**, *13*, 27–33.

Margie Patlak is a freelance science writer based in the Philadelphia area.