Spelling Out a Cancer Gene

A single mutation makes the difference between a normal gene in a human cell and a gene that causes tumors. Many cancers appear to involve altered forms of basic cellular genes.

By JULIE ANN MILLER

The dramatic difference between a normal cell and one that is malignant may be attributable to a very small change in the genetic material. In the gene responsible for malignancy in cells derived from a bladder tumor, the only abnormality is the substitution of one nucleotide (the basic subunit of DNA), according to the work of three independent groups of scientists. This genetic change is expected to alter one amino acid near the beginning of one cellular protein.

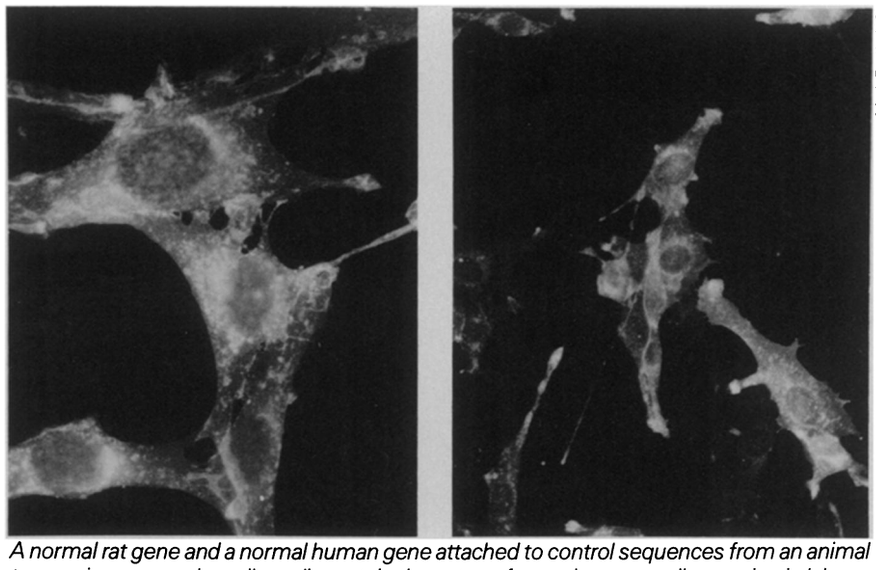
The scientists were surprised both that the genetic difference is so small and that it falls within the DNA that encodes a protein. They had expected the change to affect a region involved in turning the gene on and off. "It is hard to understand how such a simple [genetic] change can cause so dramatic a change in a cell's development," says Mariano Barbacid, one of the scientists involved.

This detailed analysis of a gene arises from the intersection of two major aspects of cancer research. One approach focuses on a group of viruses (called retroviruses) that cause animal tumors. The other work centers on the genetics of human tumor cells.

Together these approaches, with recombinant DNA and gene transfer techniques as tools, are providing a new view of cancer at a very detailed level. For certain genes a change in content, location or number of copies present in a cell has been implicated in malignancy. The question remains how to fit these genetic events into a scheme that could explain the multi-step development of human cancer.

Investigation of viruses has gone in and out of the spotlight on cancer research during the last 70 years. The first cancer-causing virus was isolated from chickens in 1911 by Peyton Rous. However, the simple idea that most human cancer can be explained as a viral infection turned out to be unlikely, and much research turned in other directions. More recently attention has returned to these viruses because they can provide clues to discovering human genes that appear to be involved in some cancer.

An irony brought viruses back into the mainstream of cancer research. Scientists have discovered that the genes contained in certain viruses — the genes that allow the viruses to trigger rapid malignant growth — had been captured from normal cells (SN: 5/26/79, p. 344). The normal cellular genes, called cellular oncogenes or, more cautiously, proto-oncogenes, are conserved over great evolutionary distances. Very similar proto-oncogenes can appear in human, rodent, bird and even fruit fly cells. The wide distribution of each of these genes (almost 20 have been identified) suggests it plays some basic role in the normal functioning or growth of a cell.



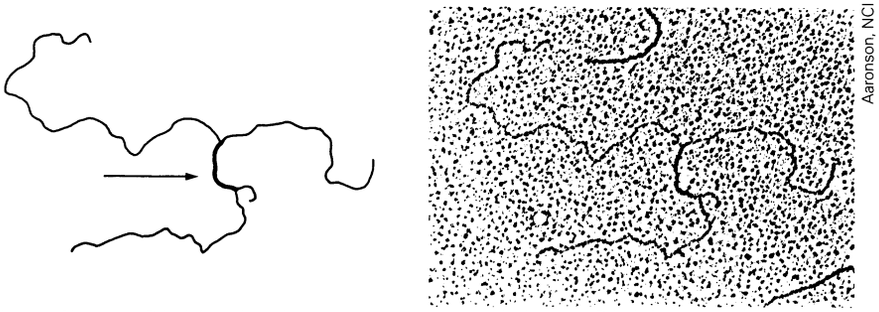
Mark Furth, NCI

A normal rat gene and a normal human gene attached to control sequences from an animal tumor virus can make cells malignant. In these transformed mouse cells growing in laboratory culture, the fluorescent stain binds to the protein product of the rat (left) and human (right) oncogene.

The gene from the normal cell not only resembles the cancer-causing gene, but in at least three cases it can be made to act in the same way. George Vande Woude of the National Cancer Institute first demonstrated that a cellular oncogene, hooked up to a control region of viral genetic material, can transform normal cells to a cancerous state. So within each animal cell are genes that can cause malignancy if they are put under a different set of controls.

Vande Woude says that this finding may explain how a variety of genetic insults are implicated in cancer. They may all change the chromosomes in such a way that an oncogene becomes active. Viruses, chemical carcinogens and other agents can alter the sequence of nucleotides either in protein-coding or control regions of chromosome. Or they can move pieces of DNA within or between chromosomes. Such changes, he says, may directly activate an oncogene or turn on its control switch. They may also move a gene away from its normal control elements, putting it under an inappropriate set of controls. Thus, activated oncogenes may underlie a variety of malignancies.

A distinct line of cancer research begins not with viruses but with human cells that have already turned malignant. In most cases of human cancer, no virus seems to be involved. Robert A. Weinberg at Massachusetts Institute of Technology, Michael Wigler at the Cold Spring Harbor Laboratory and Geoffrey M. Cooper at Harvard Medical School found that DNA taken from certain malignant cells can transform normal cells into cancerous ones, without the influence of any viral genetic material. The DNA taken from the malignant cells thus contains human cancer genes, generally called transforming genes or, again, oncogenes (SN: 9/26/81, p. 199).



Aaronson, NCI

A piece of human DNA (on right in electron micrograph) binds to DNA from a rodent tumor virus. They attach at the region (arrow), 650 paired nucleotides in length, of the oncogene they hold in common.

At least six such transforming genes have been identified. NCI's Barbacid reports that the same oncogene may be present in clinically unrelated tumors, for instance a lung tumor and an embryonic tumor. And tumors with the same clinical diagnosis may contain different oncogenes. Barbacid finds a transforming gene in about 15 percent of human tumors.

The viral and the tumor gene approaches to cancer research were recently brought together by a striking finding. The transforming genes from some human cancers match up with the oncogenes of animal cancer viruses. For example, the transforming gene of the cell line derived from a human bladder cancer is very similar to that of one rodent virus (Harvey murine sarcoma virus) and the transforming gene of several lung and colon cancers is closely related to another rodent virus (Kirsten murine sarcoma virus). These results were reported last spring by researchers at NCI, Harvard Medical School and Cold Spring Harbor.

"The most obvious consequence of these newly discovered homologies is a realization that at least some of the cellular sequence [genetic material] can become activated via one of two routes: either by recombination with a retroviral sequence, or by nonviral, somatic mutational events," Weinberg says in the August 1982 CELL.

Close examination is underway of the 20 or so distinct cancer-causing genes discovered in animal viruses and in malignant cells. Some surprising similarities have already been observed among genes that had been thought to be unrelated. M. Yoshida and colleagues at the Cancer Institute in Tokyo find, for instance, that two avian sarcoma virus genes (called *yes* and *src)* code for proteins that are homologous in 70 percent of their amino acid subunits. A third gene, called *mos,* found in a rodent virus, also seems to fall in this group.

In another group are at least four oncogenes also very similar to each other. This group includes the oncogenes identified in the bladder cancer cell line and in lung and colon cancers. These genes are thought to represent a second family, called *ras,* arising from genes that duplicated before establishment of the modern animal phyla. Esther H. Chang and Douglas R. Lowy of NCI demonstrated that normal human cells contain four different members of this gene family, and other investigators have detected a fifth member.

If the 20 oncogenes fit into a few distinct groups, the problem of discovering their normal cellular functions is simplified. Only a small number of distinct enzymatic functions may be represented by the numerous cancer genes. For one group of oncogenes scientists already know that its product is a tyrosine kinase, an enzyme that chemically alters other proteins by adding a phosphate group to the amino acid tyrosine (SN: 5/26/79, p. 344). Another group of oncogenes encodes a protein of 21,000 dalton molecular weight that binds guanosine nucleotides. Weinberg comments, "Maybe the present oncogene jungle is not so impenetrable after all."

An important question is just how the cancer-causing and normal oncogenes differ. Althogh they are almost identical in structure, there is a clear biological difference. In the cases studied, the normal gene, isolated and produced in quantity but unassisted by viral control regions, is far less effective than the gene from a malignant cell in transforming normal cells.

Scientists from MIT and NCI set out to determine the exact difference in the case of the bladder cancer gene studied. They made exchanges of defined segments of the normal gene and the one derived from malignant cells to determine a small region that could confer on a normal gene the ability to trigger malignancy.

In separate laboratories at NCI Ravi Dhar and E. Premkumar Reddy then determined the sequence of nucleotide subunits in this region of the gene. The single essential difference found among the gene's 4,600 nucleotide pairs was a sequence of GGC in the normal gene and GTC in the gene from malignant bladder cells. This change is expected to insert a valine instead of a glycine near one end of the protein encoded by this gene.

In his most recent work, Dhar found the altered gene in cells taken directly from a bladder tumor of a patient. He told SCIENCE NEWS that in this case the altered gene was also detected in blood and other apparently normal tissues of the patient, so the mutation either was inherited or occurred early in embryonic development.

The related gene in three animal cancer viruses also differs from the normal cellular gene at the same location. In each virus the glycine of the protein encoded is replaced with a different amino acid.

This work was performed by three groups. They include Weinberg at MIT, Edward Scolnick now at Merck & Co., Inc., and Lowy, Chang and Dhar at NCI; Barbacid and Reddy at NCI; and Wigler at Cold Spring Harbor.

The scientists were surprised to find the change in such a protein-coding sequence; they had thought it more likely that the change would affect regulation of the quantity of a protein produced. In virally transformed cells production of protein encoded by the viral oncogene is usually greater than production of protein encoded by the homologous gene in normal cells. This observation had suggested that viral genes transform cells by producing proteins in abnormal quantities. But in the bladder gene case there is a distinct change in the protein, perhaps like the shape change caused by a single nucleotide alteration in sickle cell anemia.

There is recent evidence for changes other than in nucleotide sequence being involved in malignancy. In work on leukemic cells two groups of scientists have found that the number of copies of an oncogene is higher in leukemic blood cells from a patient with promyelocytic leukemia than in cells of normal people. The oncogene, called *myc,* was first identified in a retrovirus infecting chickens.

Steve Collins of the Seattle Veterans Administration Hospital and Mark Groudine of the Fred Hutchinson Cancer Research Center in Seattle report that *myc* is present in multiple copies in a cell line derived from a patient's blood cells. Robert C. Gallo and colleagues at NCI estimate 16 to 32 copies of the gene are present in the same cell line. Gallo also observes extra copies of the gene in cells taken directly from peripheral blood of the patient. However, when they looked at other types of leukemia, the scientists did not find evidence of *myc* amplification.

...GTG GGC GCC GGC GGT GTG GGC...

...GTG GGC GCC G**T**C GGT GTG GGC...

A change of one nucleotide out of thousands is the essential difference between a normal human gene and one that makes cells malignant.

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Chromosomal rearrangement and translocations also seem to have a role in causing cancer. Such gross genetic changes have been linked to many cancers (SN: 9/4/82, p.151; 10/31/81, p. 278). An exciting finding reported by two groups last month links a chromosome change with an oncogene. Carlo Croce of the Wistar Institute in Philadelphia and Gallo of NCI have located the *myc* gene on human chromosome 8 in a position that suggests it may be involved in the human immune system cancer called Burkitt's lymphoma.

The hallmark of Burkitt's lymphoma is a chromosome rearrangement in the tumor cells. A part of chromosome 8, apparently including the *myc* gene, is transferred to chromosome 14 (or less frequently to chromosome 2 or 22). At the annual Bristol-Myers Symposium on Cancer Research, held at the University of Chicago Cancer Center, Croce reported that in lymphoma patients the *myc* gene is present on the altered chromosome 14. Similar work was reported there by Rebecca Taub and Philip Leder of Harvard University.

"This finding argues strongly for a role of *myc* in Burkitt's lymphoma," Gallo says. He speculates that in its new position on chromosome 14, the oncogene is expressed at an abnormally high level. The researchers find no correlation between the *myc* gene and the Epstein-Barr virus, which is implicated in Burkitt's lymphoma by other lines of evidence. They suggest that *myc* and the virus may be involved in separate steps of cancer development.

Scientists are now looking for other human cellular genes homologous to oncogenes described in work on animal viruses. Using a radioactively labeled copy of an oncogene to bind to its counterpart on the human chromosomes, Stuart A. Aaronson and NCI colleagues have located the rodent virus oncogene called *mos* on a human chromosome. They identified the chromosome by examining hybrid cells having some human and some Chinese hamster chromosomes. By the pattern of oncogene binding, they report in the September PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES "an unambiguous assignment" of the *mos* oncogene to human chromosome 8.

While feeling satisfaction with the tremendous progress made in the last two years by the application of recombinant DNA and gene transfer techniques to the study of tumor viruses and oncogenes, most scientists feel they are still a long way from explaining human cancers. Barbacid says, "Demonstration that a single genetic alteration is sufficient to confer neoplastic properties on a normal cell is in apparent conflict with the lengthy development of most human cancers." He suggests these genetic events might play either an early, common, potentially reversible role or a late, irreversible role in the multi-step process of human cancer. ☐