Another animal model, mouse leukemia virus, affects the spinal cord without causing much inflammation and causes a vacuolar myelopathy similar to that which occurs with the AIDS virus. But in mice, vacuoles (abnormal spaces) occur in the gray matter, in contrast to the changes in white matter seen with AIDS. Nevertheless, mice may be useful models because, as with the vacuolar myelopathy seen in AIDS, "you see almost a degeneration of the nerve tissue without a lot of specific cell death," says Johnson.

In human AIDS, Price thinks that the localization of virus is "more regional than structure-specific." He does not believe the virus targets one particular area of the brain for attack. Instead, he proposes that, "if the virus comes in through the brain ventricles, then it would be more likely to infect the brain regions around the ventricles," including the deep white and gray matter.

There is no direct evidence that the AIDS virus enters the brain by this route, but the virus can be cultured from CSF. This makes entry through brain ventricles, and the highly vascularized choroid plexus that surrounds them, a possibility. The fact that visna virus in sheep seems to infect ependymal cells in the choroid plexus adds some credibility to this proposal.

Even with the new information, critical questions—how does the AIDS virus enter the brain, exactly what cells does it infect, and how does it cause dementia—are still in very early stages of being answered. Complicating the issue further are the variety of infections to which AIDS patients are susceptible because the virus suppresses their immune response.

A little over a year ago, George Shaw, of the National Cancer Institute (NCI), and his collaborators reported that the brains of 5 out of 15 individuals with AIDS and brain abnormalities had DNA or RNA specific for the AIDS virus. This report indicated that the dementia associated with AIDS may be due to a specific infection of the brain, rather than a secondary effect of other infections that frequently accompany AIDS.

Working independently, Jay Levy and his colleagues at the University of California School of Medicine in San Francisco, and David Ho at Massachusetts General Hospital and his collaborators, cultured the AIDS virus from cerebrospinal fluid and from central and peripheral nervous system tissues of patients with AIDS. These studies added to the evidence that the AIDS retrovirus directly infects the brain.

Lionel Resnick of NIH and his collaborators found that the CSF of 22 of 23 patients with AIDS (21 of whom had neurological symptoms), contained antibodies specific for AIDS virus antigens. Although antibodies from the blood can enter the cerebrospinal fluid, the Resnick group calculated that much of this antibody was made inside the blood-brain barrier, indicating that the virus infected the central nervous system specifically.

It seems likely that researchers will continue to debate whether or not the AIDS virus is neurotropic and attacks brain cells specifically. Price and Johnson think it is neurotropic and Fauci reserves opinion because he has no evidence either way. Perhaps this question can be answered in an animal model or in cell culture.

Price and his colleagues have shown that there is a discernible pattern of neurological symptoms that occurs in most AIDS patients. But the time course of their development varies greatly among individuals and the severity of patients' symptoms does not always correlate well with the extent of detectable abnormalities in their brain tissue. Scientists will continue to wonder if these differences are due to the host, or if the virus itself also varies. ■

**DEBORAH M. BARNES**


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# The Yin and Yang of Cell Growth Control

*There is growing recognition that the development of malignant tumors may owe as much to the loss of growth inhibition as to a surfeit of stimulation*

When designing the cell, Nature generally built in a system of checks and balances that allow cellular activities to be closely regulated by opposing stimulatory and inhibitory influences. Investigators who have been trying for the past several years to decipher the mysteries of cancer have concentrated mostly on the forces—such as growth factors and oncogenes—that might actively stimulate the uncontrolled growth of cancer cells. Largely neglected until recently were the inhibitory forces that might check cell division and the development of malignancies.

The increasing interest in research on cell growth inhibition was evident at a meeting, albeit entitled "Growth Factors, Tumor Promoters, and Cancer Genes,"* that was held last month in Steamboat Springs, Colorado. Nearly 20% of the speakers focused on efforts to pin down the factors that might inhibit tumor development. Their results supported what Ruth Sager of Harvard's Dana-Farber Cancer Institute calls the "yin-yang theory of cancer"—meaning that the loss of inhibitory responses may be just as important for unleashing the malignant potential of cells as is activation of the stimulatory forces. In fact, both may be involved, which would be consistent with the view that most cancers develop slowly because several cellular changes are required.

Among the growth-inhibitory substances that are now beginning to attract a good deal of attention is, its name notwithstanding, transforming growth factor, type $\beta$ (TGF$\beta$). TGF$\beta$, which is a protein, was discovered a few years ago by two groups, one including Harold Moses and his colleagues at the Mayo Clinic in Rochester, Minnesota, and the other including Anita Roberts, Michael Sporn, and their colleagues at the National Cancer Institute

*Some sessions of the growth factor symposium, which was sponsored by Triton Biosciences and the University of California at Los Angeles (UCLA), were held jointly with a concurrent symposium on "Interferons as Cell Growth Inhibitors and Antitumor Factors," which was sponsored by the Schering Corporation and UCLA.

(NCI). It originally received its name because it stimulates the growth of fibroblasts and causes them to behave as if they have been transformed to the cancerous state.

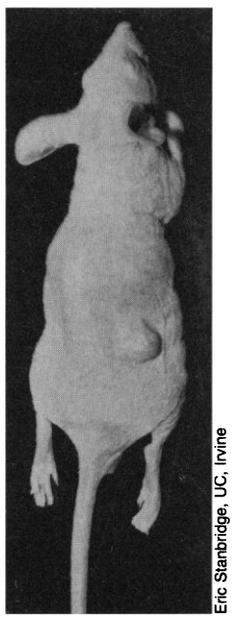
But "transforming growth factor" has turned out to be something of a misnomer. As Roberts told the meeting participants, "More often than not, TGFβ has inhibitory effects." The NCI workers and Moses and his colleagues have shown that the factor inhibits the growth of most cell types, with the exception of fibroblasts. Among the inhibited cells are epithelial cells, the keratinocytes of the skin, and lymphocytes. According to Moses, who recently moved to Vanderbilt University School of Medicine, TGFβ is very similar, if not identical, to a growth-inhibitory protein that was discovered several years ago by Robert Holley of the Salk Institute in San Diego.

Both the Moses and Sporn groups have evidence suggesting that loss of responsiveness to the growth-inhibitory action of TGFβ may contribute to the uncontrolled division of cancer cells. The active TGFβ molecule consists of two identical protein subunits, each containing 112 amino acids. Cloning of the TGFβ gene, which was accomplished last year by Rik Derynck and his colleagues at Genentech, Inc., in South San Francisco, has revealed that the 112–amino acid protein is synthesized as part of a larger inactive protein containing 391 amino acids. Assembly of the active molecule requires that the subunit be split from the larger protein and assembled into dimers.

The work at NCI and Vanderbilt indicates that some tumor cells may be defective in their ability to convert inactive TGFβ to the active form. In addition, Moses and his colleagues have identified a line of cancer cells that apparently fail to respond to the growth inhibitor because they no longer have detectable quantities of the membrane receptor to which the protein binds.

How TGFβ inhibits cell growth is currently unclear, but Moses described evidence indicating that it stimulates fibroblast growth indirectly by activating the cellular version of the *sis* gene, one of approximately three dozen oncogenes that have been identified over the past several years. Oncogenes, which cause the malignant transformation of cells at least in culture, are apparently produced when cellular genes that normally control growth and differentiation undergo some structural or regulatory alteration that causes them to malfunction. The cellular *sis* gene, for example, codes for one form of platelet-derived growth factor (PDGF). *Sis* activation by TGFβ would thus lead to a growth stimulus in the form of PDGF. The stimulatory effects of PDGF may be the result of its ability to activate two additional cellular oncogenes, namely *fos* and *myc*.

The TGFβ results illustrate what may be a common property of the molecules that regulate cell growth; the same molecule can be stimulatory or inhibitory depending on the circumstances. The same thing also applies to tumor necrosis factor (TNF), which is best known because it kills some tumor cells and is currently attracting a good deal of attention as a possible anti-cancer treatment. Nevertheless, according to Jan Vilcek of New York University Medical Center, TNF provides a potent growth stimulus for certain types of normal cells, including fibroblasts. Vilcek suggests that growth stimulation may be a major function of TNF, although he notes that not everyone would agree with him.



Eric Stanbridge, UC, Irvine

**Testing tumorigenicity.** *The nude mouse was injected in the right flank with HeLa-fibroblast hybrid cells that had lost chromosome 11 and regained their tumorigenicity. The tumor they produced is apparent. The left flank was injected with the same cells after reintroduction of a normal human chromosome 11. No tumor formed.*

In addition, Vilcek's work shows that when TNF stimulates fibroblast growth it elicits production of a β-type interferon that inhibits the division of the cells and may be part of a feedback mechanism that keeps the cell growth from getting out of hand. Growth-stimulatory and -inhibitory agents may be generally capable of inducing counteracting responses. For example, Charles Stiles of Dana-Farber and his colleagues have reported that PDGF stimulates interferon production in fibroblasts and Moses has made a similar observation with TGFβ.

What finally happens to the cell then—whether a net increase or decrease in division—depends on which of the counteracting activities gains the ascendancy. It is perhaps no wonder that a cell change that perturbs either of the opposing regulatory forces may upset the delicate balance between them and result in a cancer.

Data presented at Steamboat Springs also suggest that the anti-growth and anti-tumor effects of the interferons may be at least partly due to their ability to counteract oncogene action. For example, Robert Friedman of the Uniformed Services University of Health Sciences in Bethesda, Maryland, reported that interferon treatment can cause cells that have been transformed with the *ras* oncogene to revert to a more normal appearance. It apparently does this by inhibiting the oncogene's expression. And Adi Kimchi and her colleagues at the Weizmann Institute of Science in Rehovot, Israel, have evidence suggesting that the anti-proliferative effects of β-interferon may be the result of its ability to decrease expression of the *myc* oncogene.

In addition to examining the growth-inhibitory potential of agents such as the interferons, TGFβ, and TNF, all of which have been well characterized biochemically by now, investigators are trying to identify directly cellular genes that can suppress the growth of malignant tumors in animals. Although no tumor-suppressive genes have as yet been isolated, several investigators described evidence indicating that such genes exist and that their loss may be one of the steps required for cancer development.

Much of the evidence in question comes from studies in which cells that have undergone malignant transformation are fused with normal cells. According to Eric Stanbridge of the University of California at Irvine, the hybrids formed by fusing human cancer cells with normal human cells do not produce tumors when they are implanted in nude mice, although the cancerous parent cells do. The result implies that the normal cells contain genetic information that suppresses the tumor-forming behavior.

The Stanbridge group has spent the past few years trying to track down the gene or genes that suppress tumorigenicity. To do this they have applied information gleaned from the early cell fusion studies, which often gave conflicting results. Sometimes the hybrids were not capable of forming tumors; other times they were. What was happening, investigators eventually learned, is that some of the hybrid cells, especially those made by fusing cells of different species, were regaining their tumorigenicity because they rapidly lost the chromosomes with the suppressive genes.

Hybrids between cells of the same species, such as the human-human hybrids studied by the Stanbridge group, lose chromosomes very slowly. Occasionally, however, a hybrid does become tumorigenic again by shedding a chromosome that contains a suppressive gene. The Irvine workers have recently correlated the loss of a particular chromosome—chromosome 11—with the restoration of tumor-forming ability in hybrids

between HeLa cells, which originated from a human cervical cancer, and human fibroblasts. Moreover, they have shown that reintroduction of chromosome 11 by itself is sufficient to suppress again the hybrid's tumor-forming potential. "What all this means is that we are beginning to focus on genes on single chromosomes that are tumor suppressive," Stanbridge says.

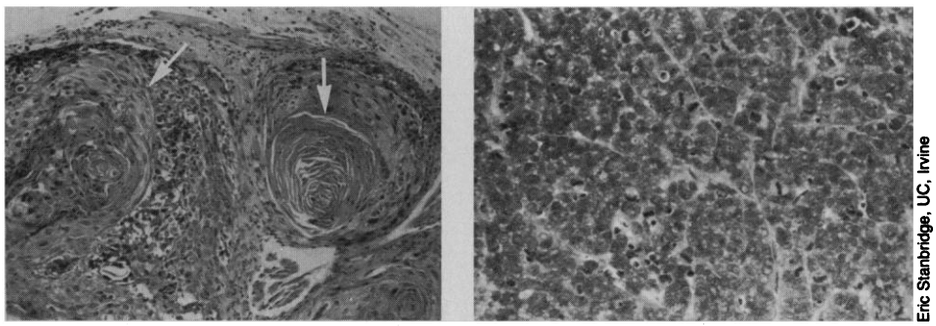
The correlation of tumor suppression with chromosome 11 is especially interesting in view of findings by Webster Cavenee, Marc Hansen, and their colleagues at the University of Cincinnati School of Medicine on the gene changes underlying certain childhood cancers. Patients with the hereditary growth disorder called Beckwith-Wiedemann syndrome have a high risk of developing any of three cancers, Wilms' tumor of the kidney, a liver cancer (hepatoblastoma), and a muscle cell cancer (rhabdomyosarcoma).

The Cincinnati workers have found that in all three types of tumors both members of the chromosome 11 pair carry a particular mutant gene even though only one chromosome 11 in normal cells from the patients carries the mutation. The implication is that the inheritance of a single copy of the mutant gene increases the patients' susceptibility to the cancers but that cells must undergo a second change, the loss of the good gene copy, before the malignancies can develop.

Cavenee suggests that the normal chromosome 11 gene is needed for cells to differentiate properly, and therefore to stop dividing. Cells from which the gene is lost completely end up locked in an immature, dividing state. "The tumors look like normal embryonic tissue, but there is a lot of it and at the wrong time," Cavenee points out.

Stanbridge's results are consistent with the idea that tumorigenicity may result from loss of a gene needed for differentiation. The hybrid cells that do not form tumors when transplanted into nude mice initially divide but then differentiate. Moreover, the hybrids acquire the characteristics of the normal cell, no matter what the nature of the cancerous cell. For example, hybrids of HeLa cells and keratinocytes differentiate into keratin-producing cells.

It remains to be seen whether the tumor-suppressive action that Stanbridge and his colleagues have associated with chromosome 11 is the work of the same gene implicated in the genesis of Wilms' tumor and the other cancers. However, Stanbridge and Bernard Weissman, who is at the University of Southern California in Los Angeles, have preliminary results suggesting that introduction of chromosome 11 into a line of Wilms' tumor cells suppresses their tumorigenicity.



Eric Stanbridge, UC, Irvine

**Nodules formed by tumor cell hybrids.** *On the left is a micrograph of a nodule formed when cells of a nontumorigenic hybrid between HeLa cells and human keratinocytes are injected into nude mice. The hybrids undergo terminal differentiation, forming keratin-filled cysts (arrows). Occasionally nontumorigenic hybrids regain their tumor-forming capacity, a change that correlates with the loss of chromosome 11. The right micrograph shows the nodule of undifferentiated carcinoma cells formed by a revertant line of HeLa cell–keratinocyte hybrids in nude mice.*

Another indication that loss of tumor suppression contributes to the development of malignancies comes from Carl Barrett and his colleagues at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina. Complete transformation of normal cells to malignancy has been shown to require at least two steps. First they must be "immortalized," that is, made capable of dividing indefinitely in culture. This can be achieved either by carcinogenic chemicals or by certain oncogenes, of which *myc* is the prototype. Then the immortalized cells must undergo a second change that makes them capable of forming malignant tumors in animals. This change can be brought about by members of another group of oncogenes, for which *ras* is the prototype.

When Barrett and his colleagues fused chemically immortalized Syrian hamster cells with tumorigenic hamster cells, they found that some of the immortalized cells suppressed the tumorigenicity whereas others did not. Moreover, the cells with the suppressive capabilities themselves resisted conversion to tumorigenicity by the introduction of *ras*. The results imply that complete transformation with acquisition of tumor-forming ability requires the loss of the suppressive function.

In another set of experiments, the Barrett group showed that complete transformation of hamster cells by *myc* plus *ras* is consistently associated with the loss of one copy of chromosome 15. Whether that chromosome is involved in tumor suppression is currently unclear, but most investigators expect that many genes with tumor-suppressive capabilities will turn up. There are after all more than 30 oncogenes.

Exactly how tumor suppressive genes might work is not yet known. As already mentioned, some may be needed for normal differentiation. Conceivably they might directly counter oncogene action, although if they do this, it does not necessarily have to be by inhibiting transcription of the genes into messenger RNA's or translation of the messenger RNA's into protein. Barrett, Sager, and Stanbridge have all shown that they can demonstrate tumor suppression even when the *ras* gene is actively making its protein product.

Although most investigators have used cell fusion methods for detecting genes that might suppress tumor formation or cell growth, Bruce Howard and his colleagues at the NCI are taking a different tack. They are trying to identify genes that slow cell growth by transferring DNA from quiescent, nondividing cells into cells that are dividing. DNA transfer methods have been highly successful in identifying oncogenes, but finding the rare cell that has had its growth slowed by acquisition of foreign DNA is inherently more difficult than finding the rare cell that has been transformed and is rapidly dividing.

Nevertheless, the results of the gene transfer experiments provide some evidence for the existence of growth-inhibitory genes. "DNA from quiescent cells is very strongly inhibitory for HeLa cells," Howard says, although he is careful to point out that the experiments do not definitively prove that the DNA contains a growth-inhibitory gene. Some toxic agent might have slowed the HeLa cell growth, although no such effect was noted with control DNA's. The NCI workers are now trying to clone the gene or genes responsible for the growth inhibition.

Eventual cloning of tumor-suppressive genes is also the goal of the investigators doing that work. Then research on the factors that inhibit cell growth and tumor formation might proceed on a more equal footing with the research on the stimulatory factors. ■ JEAN L. MARX