Cell Growth Control Takes Balance

The uncontrolled division of cancer cells may result either from excessive growth stimulation or deficient growth inhibition

DURING the past few years, a marked shift in emphasis has occurred in growth control research. For a long time, investigators concentrated almost exclusively on agents that stimulate cell growth, especially on the cancer-causing oncogenes and their products. Neglected were the genes and factors that might inhibit cell growth, partly because they are harder to identify than oncogenes, which act positively to make cells malignant. As Robert Weinberg of the Whitehead Institute in Cambridge, Massachusetts, points out, "We really only know about these [inhibitory] genes when they're not there. This is a much more difficult problem."

The tide has begun to turn, however, enough so that an entire symposium on "Growth Inhibitory and Cytotoxic Polypeptides"* could concentrate on the negative aspects of cell growth control. A major theme of that meeting was balance—cells are normally controlled by the opposing effects of growth stimulatory and growth inhibitory factors. "Cell proliferation is probably regulated by both positive and negative effects. That gives more effective regulation," says Harold Moses of Vanderbilt University, who organized the meeting with Peter Lengyel of Yale University and Charles Stiles of Harvard's Dana-Farber Cancer Institute.

When the balance between the positive and negative regulation is upset, however, the result may be the uncontrolled growth of cancer cells. Several researchers have now found that the development of a variety of cancers may be influenced by a deficiency in growth inhibition, as well as by an excess of growth stimulation. When cells escape from growth inhibition, they lose a brake that might otherwise counteract the stimulatory effects of growth factors. The new findings have potential implications for developing drugs for cancer chemotherapy.

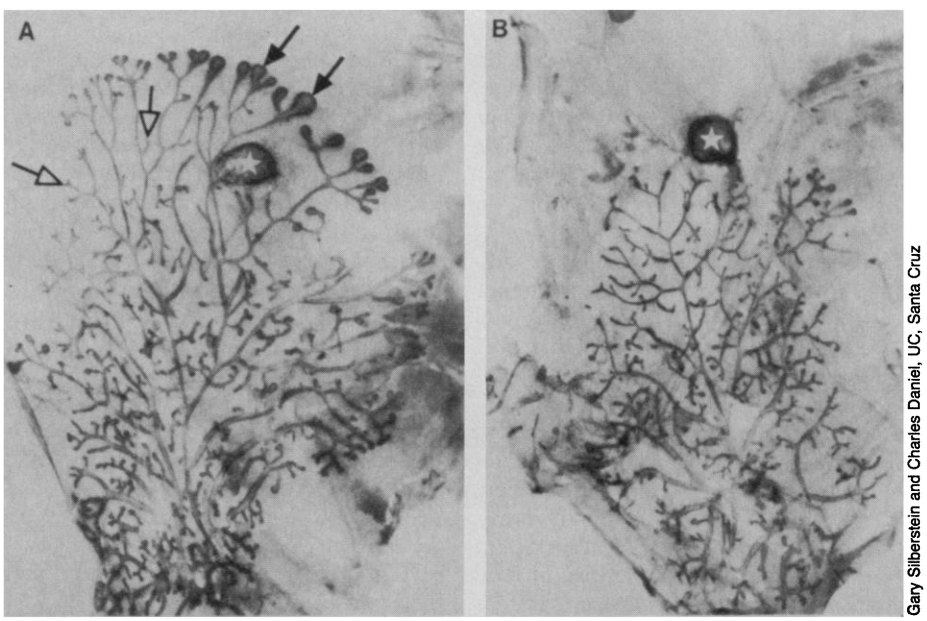
Breast cancer is one of the malignancies in which loss of negative growth influences may play a role, according to Marc Lippman of the National Cancer Institute (NCI). Many breast cancers, especially those in the early stages, grow only in the presence of estrogen hormones. Evidence obtained by Lippman and his colleagues suggests that the hormones stimulate the cells to secrete growth factors, including platelet-derived growth factor and transforming growth factor-α (TGF-α).

"Human breast cancer cells produce a wealth of growth-promoting activities," Lippman says, "but we were interested in the opposite question. Do they produce growth inhibitory substances?" The answer turned out to be yes, and the Lippman group identified transforming growth factor-β (TGF-β) as one such agent.

Although TGF-β was originally identified as a growth-stimulatory factor for fibroblasts, apparently similar to TGF-α, researchers soon learned that the two agents are structurally unrelated. Whereas TGF-α is a classic stimulator of cell growth, TGF-β turned out to inhibit the division of most types of cells, with the exception of fibroblasts (*Science*, 30 May 1986, p. 1093). The supposition then was that failure to produce TGF-β or a loss of responsiveness to its inhibitory effects might contribute to cancer development. That hypothesis is now being borne out.

As is generally the case for cancer development, several steps are required to convert normal breast epithelial cells to fully malignant cells. Lippman and his colleagues have devised a model for studying how this might occur and find that diminished susceptibility to TGF-β may be involved.

The researchers use mammary epithelial cells that Martha Stampfer of the Lawrence Berkeley laboratory prepared from breast tissue removed during breast reduction surgery. These epithelial cells appear normal, although Lippman notes that they may not necessarily be so in view of the fact that they were obtained from tissue removed from overdeveloped breasts.



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TGF-β's stops the growth of normal mammary ducts. *In the control (A), the bulbous end buds (closed arrows) of the growing mammary ducts of a young mouse have passed an implant (starred) of bovine serum albumin. The open arrows point to the blunt-tipped ends of nongrowing ducts. In (B), an implant of TGF-β blocks the growth of the ducts. They now have the typical appearance of quiescent ducts.*

*The meeting, which was held in Keystone, Colorado, on 24 to 30 January, was cosponsored by Genentech, Inc., Smith Kline & French Laboratories, Triton Biosciences, Inc., and the University of California, Los Angeles. Some of the sessions were held jointly with those of a second symposium on "Growth Factors and Their Receptors: Genetic Control and Rational Application."

In any event, the cells are first treated with a chemical that immortalizes them, that is, causes them to grow indefinitely in culture. Then a series of oncogenes are sequentially introduced into the cells to mimic the stepwise development of malignancy. "The normal, immortalized, and progressively transformed cells all produce TGF-β and have receptors for it," Lippman says, "but there is a progressive loss of TGF-β responsiveness." What causes this loss is currently unknown, but the deficiency presumably occurs after TGF-β binds to its receptor, either in the pathway for transmitting that signal to the cell interior or in the response machinery itself.

Breast cancer patients who have estrogen-dependent tumors may be treated with drugs that block the hormone's effects. The Lippman group finds that these anti-estrogens increase TGF-β production, an effect that may contribute to their ability to inhibit tumor growth. In contrast, the estrogens themselves decrease TGF-β production.

Anti-estrogen therapy often fails eventually because the tumors of patients who have been treated with the drugs become resistant to them. This resistance may reflect the loss of the ability to make or respond to TGF-β. Lippman and his colleagues have identified one line of drug-resistant breast cancer cells that no longer synthesizes the agent and another that has lost the cellular receptors to which TGF-β must bind in order to produce its effects.

Although all this suggests that stimulating TGF-β's production may benefit cancer patients, Lippman and his colleagues made one observation that indicates that this is not necessarily the case. "Cancers do not kill simply by growing. Cancers kill by invading other tissues," Lippman explains. "To our surprise and to our dismay, we found that some anti-estrogens stimulate invasion." This effect of the drugs also depends on TGF-β action.

Moreover, TGF-β has a number of additional activities that may foster tumor growth. For example, Michael Sporn, Anita Roberts, and their colleagues at the NCI find that it increases the production of connective tissue proteins, such as fibronectin and collagen, and also promotes the growth of new blood vessels. Although these actions are necessary for normal wound-healing, they may also contribute to tumor formation, if not adequately controlled. Sporn quotes Harold Dvorak of Harvard Medical School as describing tumors as "wounds that do not heal."

Lippman emphasizes, however, that his group's results, which were obtained with cultured cells, do not mean that the anti-estrogens should be abandoned as a breast cancer therapy. "If you use these drugs in patients, overall the results are beneficial," Lippman says. "Whether they would be more beneficial without the negative effects we find in vitro remains to be seen."

Breast cancer cells are not the only ones regulated by a balance between positive and negative growth control factors. Moses and his colleagues previously found, for example, that keratinocytes, skin epithelial cells, produce both TGF-α, which stimulates their growth, and TGF-β, which inhibits it. Curtis Harris of NCI has obtained similar results with bronchial epithelial cells, the cells from which lung carcinomas develop. "There's a very strict balance between growth and terminal differentiation pathways," Harris remarks, "but more interesting is the disregulation that occurs during the carcinogenic process."

Harris is using a model, similar to that developed by Stampfer, Lippman, and their colleagues for breast epithelial cells, to study the stepwise conversion of the bronchial cells to the malignant state. His results again indicate that escape from growth inhibition, possibly mediated by TGF-β, contributes to cancer development. "In normal cells, the negative growth factors seem to be dominant over the positive growth factors," Harris says, "but this is lost in the preneoplastic and cancer cells." In a similar vein, Francis Ruscetti of NCI's Frederick (Maryland) Cancer Research Facility finds lymphoid leukemia cells are not inhibited by TGF-β, whereas their normal counterparts are.

Retinoblastoma is still another cancer in which escape from the inhibitory control of TGF-β may play a role, according to results presented by Weinberg in his keynote talk at the meeting. In collaboration with Adi Kimchi of the Weizmann Institute in Rehovot, Israel, and Joan Massagué of the University of Massachusetts Medical School in Worcester, Weinberg has shown that cultured retinoblastoma cells are insensitive to TGF-β. "Normal retinal cells stop dead in their tracks when treated with TGF-β," Weinberg explains. "Retinoblastoma cells couldn't have cared less. They went on their merry way."

The investigators were able to pinpoint the cause of the change. They found that six of the seven retinoblastoma cell lines examined had lost their TGF-β receptors. This deficiency is highly unusual because the receptors are routinely found on all types of cells. The receptors of the seventh cell line were present, but abnormal.

Retinoblastomas are caused by mutations that inactivate both copies of a gene that may encode a protein with tumor suppressing capabilities. The affected gene has been cloned and its nucleotide sequence determined. Comparisons of the predicted amino acid sequence of the corresponding protein with those of other proteins have not revealed any similarities, and the function of the retinoblastoma gene still remains a mystery.

It would be a very neat story if it encoded the TGF-β receptor, but that appears to be extremely unlikely. According to Wen-Hwa Lee of the University of California, San Diego, the gene product is localized in the cell nucleus, whereas the outer cell membrane is the site of the TGF-β receptor.

Whether there is a relation between the loss of the retinoblastoma gene and the lack of expression of TGF-β receptors in the tumor cells remains to be established. In fact, Weinberg cautions, "We don't have any proof that the receptor loss is relevant to retinoblastoma." But if it is, then one possibility is that the product of the retinoblastoma gene is needed for the expression of the receptor gene. This would be consistent with the nuclear localization of the retinoblastoma gene product.

Resolving these issues should be facilitated by the isolation of the TGF-β receptor gene, an effort that will be aided by the discovery that retinoblastoma cells lack the receptor. Massagué, for one, has been attempting to clone the receptor gene for some time but has been hindered by the previous inability to find cell lines in which the receptor protein is not made. Comparing messenger RNA patterns in cells that make a particular protein with those of cells that do not make it is one way of zeroing in on the messenger RNA corresponding to a specific gene, and thus on the gene itself.

How TGF-β inhibits cell growth is incompletely understood, but indications are that it may act by altering gene expression. "The best evidence now is that it is shutting off genes by some unknown signal transduction mechanism," Moses says. He and his colleagues have found, for example, that it turns off the *myc* gene in the cells that it inhibits. The *myc* gene is activated by a variety of growth-stimulatory signals and blocking its expression may be a way of countering growth stimuli. In contrast, in fibroblasts, which are stimulated to divide by TGF-β, the agent turns on the *sis* gene. This gene encodes platelet-derived growth factor and its activation may therefore lead to cell division.

TGF-β is only one of several growth-inhibiting agents that were discussed at the growth inhibition meeting. The others include the interferons and tumor necrosis factor. All these agents, together with those that stimulate cell division, form an intricate network for regulating cell growth and differentiation. ■ JEAN L. MARX