Mice Inherit Tumor Caused by Hybrid Cancer Gene

The recent discovery that certain genes, normal constituents of normal cells, can malfunction and lead to cancer has scientists racing to discover just how these "cellular oncogenes" go haywire. By replacing the genetic "on-off" switch of one such gene, called c-*myc*, Harvard University scientists are now able to control when and in what tissues it causes tumors in mice—a critical step in learning precisely how this gene, and perhaps other cancer genes, function.

Speaking last week in Boston at the third international NATURE conference on the molecular biology of cancer, Philip Leder of Harvard Medical School described his group's series of genetic engineering experiments that allowed them first to control c-*myc*'s function in cells in culture, and finally to manipulate the gene's expression in mice. The gene c-*myc* plays a role in the human cancer Burkitt's lymphoma, in which a chromosome rearrangement moves c-*myc* to a site normally involved in antibody production.

Once the scientists had located the part of the gene likely to control its expression, the "upstream" segment containing the "on-off" switch or promotor, "it seemed logical to go ahead and begin to deliberately alter this region," Leder says. "The idea behind these experiments was that we could remove what we felt was the [gene's] normal control region, and substitute for this a control region which we ourselves could induce from without," he explains.

The normal, intact c-*myc* gene is activated by a protein growth factor called PDGF (platelet derived growth factor). Could c-*myc* be freed of dependence on PDGF, and brought under the control of some other factor, by simply replacing its regulatory region?

To answer this question, Timothy Stewart in Leder's lab constructed a set of "fusion genes." In some of these genes he removed c-*myc*'s entire regulatory region (which contains the promotor) and replaced it with the complete promotor taken from mouse mammary tumor virus (MMTV). This is analogous to replacing the ignition switch on a car so that a new type of key is needed to start it. The MMTV promotor is activated by both lactogenic hormone (which stimulates milk production) and glucocorticoid hormones. The scientists expected the hybrid gene, c-*myc* with the MMTV promotor, to be activated in the presence of these hormones. When mouse "3T3" cells carrying the fusion gene were given the glucocorticoid hormone hydrocortisone in culture, the hybrid gene's activity, as measured by RNA transcription, jumped sevenfold, showing that the fusion gene could be controlled "from without." Leder and his colleagues reported the results of these and related experiments in the Aug. 23 NATURE.

In order to study the fusion gene's behavior in animals, Stewart microinjected copies of the "decapitated" c-*myc* gene carrying the MMTV promotor into just-fertilized mouse eggs. He established from these cells 13 strains of "transgenic" mice harboring various versions of the hybrid gene in all of their cells, including the germline (sperm and eggs).

Last February, one of these genetically engineered mice, which was pregnant, developed a rare breast tumor called mouse mammary adenocarcinoma. Later, one of her daughters developed the same type of tumor. "We had never seen a tumor [in any of our colonies] before, let alone a tumor of the breast," notes Leder. Examination of RNA (which is shorter for the modified gene) from these mice showed that both carried fusion genes in which the gene's normal regulatory region was completely replaced by the MMTV promotor. Since then, other offspring from these mice have developed similar tumors, and Leder, who finds these results "extremely suggestive," notes that the gene is expressed in large amounts in mammary glands of lactating mice and in the tumors, but not in other tissues. It appears, therefore, to be controlled by its new promotor, which is activated by hormones in the mouse's breast.

Leder speculates that the normal c-*myc* gene is controlled in part by a repressor molecule, probably a protein, that binds to its regulatory region and prevents it from being activated too vigorously, or at the wrong time during the cycle of cell growth. Alteration of the gene's control region makes the gene "immune to this kind of [repressor] control — it is not shut off." Precise manipulation of c-*myc*'s expression by promotor substitutions, Leder says, should help clarify the gene's normal role in the cell and the series of events that turn a cell cancerous. □

Natural genes enlisted to fight cancer

In the midst of a proliferation of scientific investigations exploring cancer-causing genes, some scientists have begun a search for natural genes that inhibit cancer growth. George Todaro of the Oncogen Company in Seattle, Wash., says he has identified two genes whose products slow the growth of tumor cells but do not affect normal cells. These genes are distinct from the known growth factors or the cancer-causing genes called oncogenes, Todaro reported last week at the Biotech84 conference in Washington, D.C.

"This approach is a reverse in the thinking about cancer," Todaro says. "We've turned around the common selection methods to ask, 'Can you find things that inhibit tumor growth?'"

Todaro identified one "anti-oncogene" from human cells and another from laboratory animal cells, although he expects both to be present in humans. He has determined the amino acid sequence of the proteins encoded by the two genes. These gene products inhibit breast, lung and colon cancer and a skin cancer called melanoma, but they are not toxic to normal cells. They are effective at low levels, about 10^{-10} molar, a concentration similar to the effective doses of known growth factors, Todaro says. The recently identified factors work synergistically with other tumor inhibitors, such as interferons, he reports.

The new tumor-inhibiting factors appear to have their own receptors on the cancer cell surface and appear not to interact with the receptors for interferons or known growth factors. "The key next step is to see how they antagonize the effect of growth factors inside the cell," Todaro says. His laboratory is now making the genes and working to produce enough of the two factors for animal experiments.

Therapeutically, Todaro expects these factors to be combined with other tumor-inhibiting substances, such as interferons. "We're counting on synergy. We don't expect any one to be the magic bullet and be completely effective by itself," he says.

The search for naturally occurring cancer-inhibiting genes may turn up a variety of inhibitory factors, Todaro suggests. Oncogenes appear to employ several different mechanisms to trigger cancer, and anti-oncogenes (Todaro proposes calling them "orthogenes") may counter each of the various mechanisms. As well as the tumor-growth inhibitors recently discovered, there are factors called interleukins that promote cell differentiation. Other anti-oncogenes may be discovered that modify cell receptors, inhibit specific enzyme activities and alter crucial binding proteins.

"For therapy, oncogenes are just a clue," Todaro says. "There is a symmetry in nature, and I can't imagine that there are only positive control signals for cell growth." —*J. A. Miller*