Investigators Explore Role of Oncogenes in Normal Growth and Carcinogenesis

(Editor's Note: Jordan U. Gutterman, MD, chief of the Biological Therapy Section, Department of Clinical Immunology and Biological Therapy, and Edwin C. Murphy, Jr, chief of the Virology Section, Department of Tumor Biology, here answer Oncologists' questions about their oncogene research and its applications to patient care. Working with them on this research project are assistant internists Mark Blick, DO, and Razelle Kurzrock, MD, both of the Department of Clinical Immunology and Biological Therapy, and research associate Gary Gallic, PhD, of the Department of Tumor Biology.)

What is an oncogene?

Gutterman: An oncogene is a gene that appears to code for protein products involved in normal growth and maturation or differentiation of all cells. By a variety of different mechanisms, oncogenes can be aberrantly expressed; this aberrant expression can be tumorigenic.

How were oncogenes discovered?

Murphy: J. Michael Bishop and Dominic Stahelin at the University of California in San Francisco around 1970 first set out to locate the transforming gene within the genome of Rous sarcoma virus (RSV), an RNA tumor virus. After finding the transforming region of the viral genome and making DNA copies of that region using reverse transcriptase, they decided to hybridize these DNA copies or probes to the cellular DNA of normal chickens and chickens infected with RSV. As expected, the probe hybridized to normal chicken DNA; unexpectedly, it also hybridized to normal chicken RNA. This finding indicated the presence of homologues of retroviral transforming genes in normal cells.

We now know that RSV contains an src oncogene that is also found in normal host cells. Cellular oncogenes, called proto-oncogenes, have a normal function in the normal cell and are present in all vertebrates and some invertebrates as far down the evolutionary scale as yeast. By elaborate cellular gymnastics similar to any recombination method, oncogenes were probably originally "kidnapped" from normal animal cells by RNA tumor viruses and became permanent components of the viral genomes. The fact that oncogenes serve no function in the life cycle of the virus lends support to this theory. This kidnapping process is called transduction.

Are oncogenes then named after the retrovirus in which they have been found?

Murphy: Yes. Src is named for RSV, for example, erb for avian erythroblastosis virus, myb for avian myeloblastosis virus, myc for avian myelocytomatosis virus, and fes for feline sarcoma virus. These are only a few. Twenty to thirty oncogenes have been found in and named for different viruses. These three-letter acronyms also apply to the same oncogenes found in the normal cellular genome. Oncogenes are simply easier to discover in viruses that are known to be tumor causing than in normal cells and, therefore, were initially named after the virus in which they were found. There may be other oncogenes that have not yet been discovered because they haven't been incorporated into retroviruses.

What distinguishes an RNA tumor virus?

Murphy: An RNA tumor virus, which may or may not contain oncogenes, is unique in its possession of an enzyme called reverse transcriptase (RT) that reverses the flow of genetic information, which normally proceeds from DNA to RNA, in cells infected by the virus. Because of this reverse action, the RNA tumor virus is also called a retrovirus. Each retrovirus contains two identical strands of RNA. RT allows the genetic instructions embodied in the viral RNA to be integrated into the DNA of the infected host cell. The host cell may then manufacture new virus particles; in addition, the DNA copy of the virus genes becomes a permanent part of the cell's genome. Those retroviruses containing oncogenes have been found to have greater tumorigenic potential. DNA tumor viruses also contain genes that cause tumors, but there is no normal host gene that corresponds to transforming genes found in DNA viruses. Unlike oncogenes of the RNA tumor viruses, oncogenes of the DNA tumor viruses have a defined function in the life cycle of the virus.

Specifically, what are some of the functions of oncogenes in normal cellular growth?

Gutterman: Some cellular oncogenes may be involved only during early embryogenesis and then are shut off, but others function continuously during the normal regulation of the cell cycle. The myc and ras oncogenes are probably quite important during normal metabolism, for example. Cellular oncogenes are almost certainly involved in differentiation. Certain oncogenes are only activated in specific tissues.
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Murphy: A recent study showed that in many different species almost all cells express the ras oncogene at all times. The p21 product of the ras oncogene contains an enzyme, guanosine triphosphatase (GTPase), that is crucial to the action of adenylylcyclase, another enzyme that generates cyclic adenosine monophosphate, or cyclic AMP. Cyclic AMP transmits signals from the outside to the inside of a cell, making it extraordinarily important for communication between a cell and its environment. The c-sis oncogene has been shown to be related to platelet-derived growth factor receptor, which is highly expressed during blood vessel formation. A number of oncogenes are known to be tyrosine kinases. Enzymes also associated with epidermal growth factor and thus key elements in growth control. Some oncogene products are known to be nuclear proteins, such as myc, myb, and fos. Fos, originally found in murine osteosarcoma virus, is highly expressed in hematopoietic tissue. Each oncogene product, whether a growth factor, a receptor, or a tyrosine kinase, most likely has only one function, as do other proteins in general.

How are oncogenes involved in carcinogenesis?

Gutterm: Tumorigenesis by retroviral infection is one mechanism we have already discussed, although this may be rare in human cancers. Retroviruses, as well as other viruses, by inserting viral elements into a chromosomal segment adjacent to an oncogene, may cause aberrant oncogene expression and result in malignant transformation. Three other mechanisms of carcinogenesis occur more frequently. One of these is chromosomal translocation in which segments of chromosomes change positions. Such an aberrancy is more common in leukemias and lymphomas. For example, in chronic myeloid leukemia (CML) a piece of chromosome 9 is translocated to chromosome 22. There is a reciprocal translocation of a piece of chromosome 22 to chromosome 9. The rearranged chromosome 22 segment is termed the Philadelphia chromosome (Ph1). We now know that the sis and abl oncogenes are on chromosomes 22 and 9, respectively, and are reciprocally translocated in Ph1-positive CML. The abl oncogene is probably involved in normal growth and differentiation in certain tissues. In Burkitt’s lymphoma, a portion of chromosome 8 containing the myc oncogene shifts to chromosome 14. The cause of the regular occurrence of such translocations is unknown. Viruses or movable genetic elements similar to transposons found in bacteria may be involved. As far as I know, in every known translocation associated with human cancer, one or more oncogenes is involved.

Another mechanism of carcinogenesis is mutation (i.e., an alteration in the structure of the genes). Mutation is common in the ras family of oncogenes. A change in certain amino acid subunits of the ras p21 protein leads to a gene product that possesses drastically reduced GTPase activity and is highly transforming. We don’t know the incidence of oncogenic mutation. By assays currently available, scientists have found that about 15% of solid tumors have a mutation in the ras oncogene that allows the gene, normally involved with growth, differentiation, or regulation, to become a transforming gene.

The third carcinogenic mechanism of oncogenes is amplification in which 10, 50, or even 100 copies of the gene are produced. Consequently, the cell is flooded with an excess amount of protein, causing the cell to divide indefinitely. Amplification of c-myc, for example, has been found in promyelocytic leukemia and in colon carcinoma cell lines.

Can a single oncogenic mechanism produce a tumor?

Gutterm: Most probably a combination of mechanisms is at work in the different stages of development of cancer. Cooperation between two or maybe even three oncogenes may be required to create a malignancy. A mutation of an oncogene may be followed by amplification of a second oncogene and then perhaps translocation of a third gene. Several such mechanisms may be involved in some connecting fashion, but the fundamental message is that a variety of molecular changes occurs in these oncogenes that causes them to lose their normal control mechanisms and so lead to malignancy.

Murphy: In culture, certain oncogenes, such as the ras genes, are able to transform cells while others, like the myc gene, are nontransforming but are able to immortalize cells. These results imply that specific oncogenes have certain functions that are involved in different facets of tumorigenesis.

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Lung Cancer Surpasses Breast Cancer as Mortality Risk to Texas Women

Since 1983 lung cancer has been claiming the lives of more Texas women than breast cancer, historically women's biggest cancer threat. In 1983 in Texas, 1,895 women died of lung cancer and 1,785 died of breast cancer.

According to Vincent Guinea, MD, chairman of UT MDAH's Department of Patient Studies, the lung cancer mortality rate for Texas women nearly doubled between 1970 and 1982, jumping from 11.9 cases per 100,000 women in 1970 to 22.6 cases per 100,000 women in 1982 and increasing from 9.7% to 17.4% the proportion of deaths from malignant neoplasms attributed to lung cancer. Breast cancer mortality rates during this same time remained stable.

Texas is one of nine states in which the number of lung cancer deaths for women has outpaced breast cancer deaths. In the United States as a whole, breast cancer is still the leading cause of death in women, but the American Cancer Society estimates that in 1986 the lung cancer death rate for women will surpass that of breast cancer to become the leading killer of women nationwide.

Two reasons account for the state's being one of the first to have the number of lung cancer deaths surpass the number of breast cancer deaths. First, Texas has a higher than average incidence of lung cancer. Scientists blame the higher rate on Texas's broad coastal area because of the environmental factors and cancer-causing exposures related to industry typically found in coastal areas. But Guy R. Newell, MD, chairman of the Department of Cancer Prevention, thinks that far more significant is the high concentration of Hispanic people in Texas. Because Hispanic women have a substantially lower incidence of breast cancer than do white women, the incidence of breast cancer is lower overall in Texas. Therefore, in Texas the lung cancer mortality rate would have a narrower than average gap to close in meeting and surpassing the breast cancer mortality rate.

Unfortunately, survival rates for lung cancer are poorer than those for breast cancer. Based on cases diagnosed 1973–1980, the National Cancer Institute reports a relative five-year survival rate of 74% for patients with breast cancer, but for patients with lung cancer the relative five-year survival rate is only 12%.

Because 85% of lung cancer cases are attributable to cigarette smoking, the increase in lung cancer mortality rates in women can be largely attributed to the increase in the number of women who smoke. During the years of World War II, the number of women smokers increased considerably, and with each successive birth cohort the number of female cigarette smokers has increased. Although the proportion of males who smoke has historically been higher than that of females who smoke, now there are more adolescent girls who smoke (12.7%) than there are adolescent boys who smoke (10.7%). Adding to this effect is the fact that fewer women than men are quitting: according to prevalence statistics, the percentage of men smoking dropped 14.4% between 1965 and 1980, but the percentage of women smoking dropped only 4.4%.

Newell predicts a very bright future potential for having a society free of smokers, and because of this he supports an aggressive preventive approach. He suggests that the physician follow the model outlined by Lichtenstein and Danaher in Chronic Obstructive Lung Disease: Clinical Treatment and Management (edited by R. E. Brashear and M. L. Rhodes): (1) be a nonsmoking, healthy life-style model; (2) supply information on the risks of smoking and their reduction after quitting; (3) encourage quitting; (4) refer patients to smoking cessation programs; and (5) recommend specific strategies for quitting and follow up to provide reinforcement.

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How is the RNA expression of a particular oncogene identified and measured in certain tumors?

Murphy: Generally, one does this by measuring the amount of radiolabeled oncogene DNA probe that can hybridize to RNA obtained from tumor tissue. In practice, total cellular RNA is prepared from the tumor tissue and the messenger RNA (mRNA) class of RNA species is isolated from this mixture. The RNA can then be separated into discrete classes by size by electrophoresis in agarose gels, transferred to nitrocellulose filters, and hybridized to radiolabeled probes derived from retroviral oncogenes or from human proto-oncogenes that have been molecularly cloned based on their homology with the retroviral.
New NCI Data Base Offers Physicians Up-to-Date Cancer Treatment Information

Physician's Data Query (PDQ), a new computerized cancer information bank, is putting National Cancer Institute (NCI) guides to diagnoses, protocols, and treatment centers at the fingertips of physicians who operate computer terminals.

Developed by the NCI and introduced to physicians by teleconference early this year, PDQ breaks down the information it offers into three categories:

- state-of-the-art information on prognosis and treatment for about 80 different types of cancers
- protocols from more than 1,000 active clinical trials
- a directory of physicians and organizations that care for patients with cancer.

Vincent T. DeVita, Jr, MD, director of NCI, introduced the system. "This is an experiment to reduce mortality," DeVita said, explaining that a major goal of the system is to save lives.

The PDQ cancer information system holds prognostic and treatment information on all major types of cancer. Each of these types is described in a capsule statement and a longer general summary. Summaries include prognosis, relevant staging and cellular classification systems, and comparable treatment options considered state-of-the-art treatment by type, stage of disease, or both.

More than 1,000 active treatment protocols, which are updated monthly, are included on PDQ. These are protocols directly supported by NCI or submitted for inclusion in the PDQ data base by clinical investigators nationwide.

About 10,000 physicians who devote a major portion of their clinical practice to treating cancer patients are listed by name, address, and telephone number in the PDQ directory, but DeVita said that being listed does not constitute an NCI endorsement. Along with the physicians are listed the names, addresses, and telephone numbers of about 2,000 institutions and health care organizations involved in cancer patient care. Both sets of names are indexed geographically.

The protocol file was written and refined with the help of more than 400 cancer specialists. Seventy-two medical, surgical, pediatric, and radiation oncologists serve as members of an editorial board and as consultants responsible for keeping PDQ up-to-date. UT MDAH's Eleanor D. Montague, MD, deputy chairman of the Department of Radiotherapy, and Oscar M. Guillamondegui, MD, deputy chairman of the Department of Head and Neck Surgery, serve as consultants.

To make PDQ available commercially, NCI has joined with the partnership of W. B. Saunders, the world's largest medical book publisher, and BRS (Bibliographic Retrieval Services), the company supplying the National Library of Medicine's MEDLINE, a medical literature on-line interactive bibliographic searching and retrieval system. BRS/Saunders calls the data base service "Colleague," and it includes other information besides PDQ, such as the full text of more than 15 books and four journals, including The New England Journal of Medicine, and abstracts and indexes, including MEDLINE.

To access PDQ, a user needs a terminal that has a screen width of at least 80 characters and a modem. Users must pay a $50 one-time registration fee, and a monthly minimum charge is levied but applicable toward usage charges. The user's fee varies depending on whether the system is accessed during prime or nonprime hours. PDQ is accessible 22 hours per day Monday through Friday and 13 hours on Saturday.

(Physicians who desire further information should write PDQ Information Coordinator, National Cancer Institute, R. A. Bloch International Cancer Information Center, Building 82, Rm. 105, 9030 Old Georgetown Road, Bethesda, MD 20205—Ed.)

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oncogenes. If a particular RNA species bound to the filter is complementary to the DNA probe being used, the DNA probe will specifically hybridize to this RNA and survive the washing procedure designed to remove unhybridized probe. X-ray film is finally exposed to the filter. The position and intensity of the signal observed will yield information on the size and amount of an oncogene mRNA in a given tissue.

Are all tumors the result of aberrant oncogenic expression?

Murphy: I don't know the answer to that, but I don't think it is coincidental that in almost every case the known oncogenes seem to be involved with chromosome translocation. Also significant is that, of the very large number of genes in the human genome, only the 25 to 30 known to be involved in malignancy are found in acutely transforming retroviruses, usually in a mutated form. Such evidence suggests that transformation may result from an upset in the normal functioning of the oncogenes. This does not suggest, however, that altered oncogenes are solely responsible for tumorigenesis.

Is information obtained from oncogene research being used at UT MDAH to treat patients?

Gutterman: We hope that eventually the answer will be yes. The therapeutic aspects of our oncogene program with fresh human tumors are beginning to come together. Mark Blick, Razelle Kurzrock, and I are specifically studying the regulation by interferon of oncogenic expression in CML, chronic lymphocytic leukemia, acute leukemias, and other tumors. Interferons that are available for use in the clinic are potent antigrowth substances and are regulatory agents of differentiation in the body. Alpha-interferon, for example, is an important inhibitor of several growth factors, including epidermal growth factor, platelet-derived growth factor, and sarcoma growth factors. Alpha- and gamma-
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interferon probably regulate different oncogenes. We are getting excellent results with alpha-interferon in the control of excess growth of white blood cells in the benign phase of Ph'-positive CML. As I said previously, translocation of the c-abl oncogene is characteristic of Ph'-positive CML. The product of the abl oncogene has a particular protein kinase activity. We're now testing whether interferon can regulate the aberrant expression of that oncogene, shut down or dampen the excess enzymatic activity that probably allows these white cells to proliferate excessively.

What other substances have you used, or do you plan to use, in the clinic to regulate oncogene expression?

Gutterman: Retinoic acid and other biologic substances like the interferons might be used to regulate the expression of certain oncogenes. Recent evidence shows that as certain tumor cell lines in culture mature in the presence of retinoic acid, the expression of certain oncogenes decreases. The same thing happens with gamma-interferon and vitamin D. But it is not clear whether these substances first allow the cells to differentiate and so shut off the oncogenes or the substances shut the oncogenes off, allowing the cells to mature normally. The myc oncogene is down-regulated (i.e., has decreased RNA expression) by alpha-interferon in a Burkitt's lymphoma cell line. These changes precede the antigrowth effects of alpha-interferon. I am sure many chemotherapeutic agents work to control oncogenes, particularly those that come from natural products, from plants like vinca alkaloids or antibiotics. Perhaps these substances are in plants for the same reason, to regulate growth and differentiation. Plants also have oncogenes and develop tumors. Though certain chemotherapeutic agents probably work through the control of oncogenes, attention is now focused on the interferons and vitamins.

Is the therapeutic use of monoclonal antibodies to turn off aberrant oncogene expression a possibility?

Gutterman: Yes, for both diagnostic and therapeutic purposes we hope to use monoclonal antibodies in our work with oncogenes. Any oncogene protein or growth factor receptor that is on the cell surface may be located or possibly regulated by monoclonal antibodies. The oncogene protein erb-B is located on chromosome 7, where the epidermal growth factor receptor is. It is, in fact, part of the epidermal growth factor receptor. But erb-B can be overly expressed in certain tumors and may be involved with excessive growth. Monoclonal antibodies to the epidermal growth factor receptor have been developed.

Have you found any parallels between a decrease in oncogene expression and tumor regression?

Gutterman: We are studying that at present. Another group may have shown this in patients with CML. The abl gene product was shut off by interferon and the patient went into remission. But there is no proof that the cause of the remission was the change in the expression of the oncogene. In fact, there is no clear evidence in patients that the change in expression of an oncogene by any agent is associated with regression of a tumor. We have results that suggest this possibility in certain tumors, as in the Daudi cell line of Burkitt's lymphoma. The myc gene is involved in the uncontrolled growth of these tumor cells in tissue culture. Scientists have used interferon to shut off the expression of the myc oncogene within about three or four hours and have observed a delay in growth a day or two later. This evidence suggests that interferon probably is involved in the shutting off of growth in tissue culture, possibly through its effect on expression of an oncogene.

Murphy: It is really not clear yet whether tumor regression necessarily involves a decrease in oncogene expression. In Gallick's and Kurzrock's work at UT MDAH with colon carcinoma, metastatic lesions in the lungs appear to have a decreased expression of ras relative to the adjacent normal tissues and the primary tumor. The ras protein expression is inordinately depressed in metastatic colon cancer lesions. This seems to suggest that the oncogene does select for a cell type. It could be that metastatic cells are no longer sufficiently differentiated in that they have lost their specific tissue type to the extent that they no longer require ras expression. Or perhaps in an early stage of the tumor, ras was overexpressed and became toxic; the only cells to survive and metastasize may have been those that produced less ras. Again, to investigate these theories we need to study the molecular events that occur.

Dr. Gutterman, you mentioned in another interview the possibility of eventually defining tumors by their molecular profile and basing treatment on this profile. Please explain briefly.

Gutterman: We are beginning to define tumors on a molecular basis, for example, in CML, lymphomas, and other tumors. If we know which oncogene or oncogenes and growth factors are involved in the growth of a patient's tumor, we may be able to use inhibitors of such oncogene products. For example, the ras gene is known to be mutated and thus aberrantly expressed in a small percentage of cancers of the colon, lung, bladder, and other solid tumors. Fifteen percent of solid tumors are thought to have a mutation of the ras gene producing an amino acid change. That mutation causes overexpression of the ras protein product p21, and overexpression of p21 is highly transforming. There is recent evidence that alpha-interferon can inhibit the production of the nonmutated protein when excessive in certain mouse tumor cells and can actually cause a phenotypic reversion. The transformation or malignant process can be reversed in tissue culture with interferon by shutting off the overexpression of this oncogene. So, theoretically, colon cancer, if partially caused by an excess transcription of the ras oncogene protein, might be reversed or controlled with interferon if the cancer were detected early enough. We don't know if this is possible in humans, but it has been shown in tissue culture and in animals.

Murphy: In Burkitt's lymphoma, the translocated myc oncogene, which in its normal location is extremely important in normal growth, has lost its normal control mechanisms and continues to be transcribed. It was reported about six months ago that beta-
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interferon is a potent inhibitor of the expression of the myc oncogene and of the growth of lymphoma cells. This natural substance stops myc expression when myc has lost its normal control mechanism, which is probably the effect of the action of another protein. This is an example of the effect oncogene research may have on the direction of cancer treatment. Rather than nonspecifically treat cancer, we are going to approach the disease on a molecular basis in the next decade.

Gutterman: I suspect that one day we will have specific inhibitors for each of these oncogenes. Each oncogene must be controlled by natural mechanisms; otherwise, cells would go awry. That is, there are natural antioncogene proteins. One day we will know the specific sequences of these substances that naturally regulate certain oncogenes and may be able to use these proteins in therapy to regulate aberrant oncogene expression. In addition, characterizing cancers molecularly should aid in diagnosis and in monitoring patients during and after treatment.

If, as you stated previously, more than one oncogene growth factor is involved in malignant transformation, how will this affect future therapy?

Gutterman: It is not clear whether these growth factors work in sequence in different stages of disease or work together. Therapeutically, we're going to have to use several antigrowth factors, such as the interferon family, either consecutively or in combination to shut off different parts of the cycle that produce aberrant oncogene expression.

Murphy: As we have learned more about the 25 to 30 oncogenes identified so far, the problem they pose has become as complex as the inside of an atom. In the past four or five years an incredible number of growth-promoting or -altering substances have been found in and among cells. Cells are communicating with themselves and each other all the time. In this complex communication network, genes are likely to be turned on and off by different substances. Oncogene research is still an extremely open field with many dark corners. It may be best to focus our study on the enormous number of environmental stresses or stimuli affecting the relatively few number of known oncogenes to understand the cellular mechanisms involved in tumorigenesis and to find the appropriate sequence or combination of substances to shut it off.

Gutterman: In the clinic we're beginning to test the first really significant combination of natural antigrowth substances with alpha- and gamma-interferon. This work is independent of the oncogene program, but in diseases whose oncogene expression we can measure, as in the chronic leukemias or other tumors of blood, we will be studying oncogene expression simultaneously.

What unique contribution is UT MDAH making in oncogene research?

Murphy: Our oncogene program is unique in that we have the clinical resources to do research more relevant to the problems oncogenes are causing in patients. We are able to study oncogene expression in fresh human tumor biopsy specimens instead of in tissue culture materials, which are easier to work with but may present an unrealistic experimental situation. My laboratory is involved in the generation, purification, and preparation of probes for 10 to 12 different oncogenes that are being used to study the expression and structure of proto-oncogenes in fresh primary or metastatic human tumor samples. We have had to overcome great technological barriers to work with fresh samples, but the advantage of this approach is that we are better able to understand the real situation in the cancer patient. As a result, we have begun to make substantial advances.

Gutterman: This collaboration between clinicians and researchers fuels progress in the laboratory and the clinic. Information obtained from oncogene research helps to identify substances, such as alpha-interferon, gamma-interferon, or retinoic acid, that may inhibit tumor growth or induce cell differentiation through effects on oncogenes. Patient response to these substances may provide a clue to understanding the complex network of communication in and among cells and to discovering tumorigenic aberrancies in this network.

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