Oncogene research: new inroads in study of cause and cure of cancer

by Ned Rosinsky, M.D.

Scientists have discovered that one of the major causes of cancer is the abnormal functioning of otherwise normal genes. A state-of-the-art symposium at the National Cancer Institute in Bethesda, Maryland, on Nov. 14-16, reported new findings in this area relating abnormal genes and abnormal control of normal genes to the processes which underly cell growth and tissue repair. These insights suggest numerous possibilities for cancer treatment and prevention.

The potential for this line of research began in the 1960s when the development of techniques for growing animal cells in laboratory tissue culture made possible the detailed study of conditions which can transform normal cells into cancerous cells. The effects of radiation, carcinogenic chemicals, chromosome breakages and abnormal reattachments, and viruses were all investigated. Viruses became a focus of intense research in the early 1970s after a number of animal cancers were traced to viral infection, since this implied that it may be possible to produce a vaccine against cancer. While only several relatively rare forms of human cancer were subsequently found to be caused by viruses, this line of research led to the isolation of the particular genes in the virus which were capable of transforming cells.

To situate the specific findings, and to emphasize that cancer involves tissue-level changes as well as genetic changes, it is helpful to have a general overview of cancer formation.

Although there are more than a hundred distinct kinds of cancer as classified by tissue typing, some aspects are common to most, if not all. There is a loss of coordination of cell growth resulting in a chaotic microscopic tissue appearance, which is usually progressive as the tumor grows. There is also an increase in the cellular division rate in many cancers, but not all.

In addition to this apparently chaotic and increased growth, there are two key processes: invasion and metastasis. Invasion requires the destruction of normal tissue surrounding the tumor and extension of the tumor into new adjacent areas. Tumors may invade any tissue, including bone. Metastasis is the separating off of parts of the tumor, parts as small as individual cells, which leave the main tumor mass and travel to distant sites in the body to start new colonies of tumor growth. Death occurs either by a mass effect of the tumor such as by blocking the lungs or intestines, or destroying the brain by pressure; by invading and destroying the major part of a vital organ such as the liver; or by generally weakening the body, including the immune system, so that infections become lethal.

A surprising result of this cancer-virus research was that the genes involved, termed "oncogenes," were either identical or nearly identical to normal genes already present in the host organism, including humans. This implied that cancer involves either an abnormal functioning of otherwise normal genes, or the slight modification of normal genes. These hypotheses were both confirmed by the malignant transformation of normal cells grown in laboratory tissue culture by the addition of DNA to the culture from cancerous cells.

Each gene is a long sequence of hundreds to thousands of nucleotide molecules which ultimately direct the production of a particular protein. Adjacent to each gene is a sequence of nucleotides which functions to determine the degree of activity of the gene; this is termed the promoter, enhancer, or activator region of the DNA.

Researchers have established that some oncogenes produce normal proteins but have abnormally functioning activators. Other oncogenes themselves are abnormal, in as small an area as one abnormal nucleotide in the entire gene. For example, the normal human gene *ras* becomes an oncogene with the change of only one nucleotide, causing the twelfth amino acid in the protein amino acid chain to change from glycine to glutamine.

Additional oncogenes which are active in human cancer but are not found in any viruses have been identified, and the total of all oncogenes is now over 20, with several new ones announced at the above-mentioned symposium. While the original concept of oncogene included an invading virus as the source of the gene, this was later expanded to include any DNA which when added to cells transforms them into cancer. At this time, active oncogenes have been found in 15-20% of human cancers, including high percentages of the common tumors of the lung, breast, and colon.

Since the oncogenes seem to be either identical or nearly identical to normal genes, and we are interested in what these oncogenes do to cause cancer, the question arises as to what are the normal functions of these genes. Preliminary studies

showed that most of the oncogenes are highly conserved in evolution, with nearly identical normal genes occurring in species as different as man, fruit flies, and yeast. This suggests that these genes are over 600 million years old, and that their normal activity is probably related to some very basic function or functions of living organisms.

Excitement over these discoveries increased last February when Dr. Steven F. Josephs and others at the Laboratory of Tumor Cell Biology at the NCI reported that the protein product of the *sis* human oncogene is a normal growth-stimulating substance called platelet-derived growth factor (PDGF), which is secreted by the microscopic blood platelets when a clot forms in the area of a wound and which stimulates the growth and multiplication of connective tissue cells, fibroblasts, which is a key part of the healing process. The *sis* gene was originally discovered in simian sarcoma tumors.

Another oncogene, termed *erb*-B, was more recently found to produce a protein which is very similar to a growth stimulus receptor found on the surface of normal epithelial cells; this receptor responds to epithelial growth factor (EGF). There is evidence that this receptor is crucial in early stages of embryological development. Twenty-five percent of human brain tumors have increased amounts of receptor to EGF on their cell surfaces. These two examples suggest that oncogenes may act at various stages of the cascade of steps involved in growth control and modulation, *sis* producing a growth-stimulating hormone-type substance, *erb*-B producing a growth hormone receptor which is situated on the surface of the target cell.

Treatment strategies

While the study of oncogenes may in the future open up new aspects of basic questions in the biology of growth and thereby suggest ways to control cancer as well as other diseases of aging, the current state of knowledge may be sufficient to make significant inroads directly. Each level of the above-referenced cascade effect is open to such intervention. At the NCI conference, Dr. David Baltimore suggested the following oncogene grouping scheme which reflects the cascade approach.

First is a series of 11 oncogenes which are similar in nucleotide sequence to the *erb*-B oncogene and which may produce other growth hormone receptors located on the surface of cells.

Second is a series including the *ras* human oncogene which appears to mediate between surface receptor activity and cytoplasmic cyclic AMP, that is, its protein product carries the activation signal into the cell.

Third is a series of nuclear-associated oncogenes typified by the *fos* oncogene which may carry the activation into the cell nucleus. The *fos* gene is normally active in mouse placenta growth, which interestingly involves not only rapid cell proliferation but also active invasion of the uterine wall in the course of normal development.

The fourth class is represented by the *sis* gene which directly produces a growth-stimulating substance.

Oncogenes may be produced by several different modes. In Burkitt's lymphoma, a lymph-cell cancer, the *myc* oncogene is not itself changed, but its piece of chromosome is broken off and reattached to another chromosome, placing the gene very close to the DNA activator area which normally controls a gene for antibodies. It seems to be this mismatch which results in abnormal production of the *myc* protein product, and the lymphoma. Interestingly, normal fibroblast cells which are stimulated by PDGF (which itself is the product of the oncogene *sis*), show an increase in the normal *myc* gene activity, again suggesting a regulatory cascade among classes of oncogenes. There are numerous examples of pairs of oncogenes being needed to transform normal cells into cancer, and this is also consistent with the finding in long-range studies of human cancer that suggests that there are at least two steps involved, induction and promotion. In another case, the cells in Wilm's tumor, a virulent childhood cancer, frequently show the loss of a piece of chromosome near a known oncogene.

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A second oncogene production mode is chemical carcinogen effects. The NCI symposium documented the discovery of two new oncogenes which were identified after exposing cells to known carcinogens. One of these, named *neu*, was isolated from a rat neuroblastoma tumor which had been induced by the carcinogen ethylnitroso urea. This oncogene is in the *erb*-B class.

A third mode is the viral effects, and here there can be either the insertion of a powerful viral promoter area near a

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normal animal or human gene, or the insertion of a variation of the animal or human gene from the virus. It is intriguing to speculate here that the virus does not "accidentally" pick up a copy of the oncogene in its self-replication in the cell and thereby spread it in an abnormal fashion, but that the incorporation of normal cell growth stimulators into the virus may itself be useful for the more rapid spread of the virus, by causing the abnormal proliferation of infected host cells. That is, what appears to us as viral-dependent oncogene activity may have arisen as part of an efficient mode of parasitism by the virus. This would, of course, be an aberrant activity of an otherwise normal or nearly normal growth-related gene.

While much remains to be learned about the functioning and production of oncogenes, the current discoveries are consistently pointing in the right direction, and the NCI has responded by substantially upgrading the research funding for this area. In 1983, 3.7% or $36 million of the $1 billion budget was devoted to pure oncogene work. This doubled to 8.4% in 1984 and will again double in 1985. Since the interest in this area began to swell in the early 1970's, a total of over $1 billion has been spent on oncogene research, including associated viral studies.

Specific treatment approaches

In the area of diagnosis, oncogenes are helping to assess tumor virulence. Oncogenes sometimes are copied multiple times in the same chromosome, and the degree of this gene amplification correlates with virulence in some tumors. Also, there are sub-types of some tumors based on the specific oncogene found; for example, in small cell lung carcinoma, 80% of tumors have active oncogene *myc*, but there are three distinct types of *myc* found in these tumors and this may correlate with response to specific therapies.

A further aid in diagnosis is the possibility of making specific antibodies to oncogenes or their protein products, attaching radioactive tracers to the antibodies, and using this complex to track down small cancers in the body which would not otherwise be found. The cancers can then be treated by either attaching toxins or powerful radiation emmiters to the antibodies, or using some other available treatment such as surgery or direct radiation from outside the body.

Antibodies to specific oncogenes have already been shown to be useful in treating tumors. The specificity required to do this is considerable, but already monoclonal antibodies have been developed which can distinguish between the normal *ras* oncogene and its malignant variant, even though the difference between the two is only one amino acid in a protein containing hundreds of amino acids.

While the oncogene work points up the close connection of the cancer process to normal processes, and this may seem to imply that treatment will be even more difficult than previously assumed since cancer cells would have to be distinguished by any treatment modality, the specificity of monoclonal antibodies may weigh heavily in the other direction, particularly since oncogene research may help to pinpoint new targets for treatment intervention.

Another treatment difficulty in this work is that some of the oncogene products may be inside the cell and therefore not easily accessible to administered antibodies, since antibodies typically do not cross the cell membrane. However, the NCI is now developing methods which have considerable promise for delivering the antibodies across the cell membrane.

Taking another line of attack, the *ras* oncogene is involved in cytoplasmic GTP activity (an analogue of ATP), and drugs which are analogues to GTP may be useful in interfering with this action. As more is learned about the metabolic activities of these regulators, more targets for treatment will become evident. This is analogous to the action of antibiotics, which interfere selectively with normal bacterial cell functions, such as the production of the cell wall needed for bacteria to grow. As the examples of antibody usage suggests, the most efficient treatments and prevention strategies may ultimately be to induce the body's own immune system to more efficiently control the cancer process. There is much evidence that the immune system normally does this in a variety of ways, and the increasing rate of cancer in the aged population correlates well with the decline of the immune system.

Oncogenes are nearly identical in man, fruit flies, and yeast, and so, perhaps over 600 million years old, and probably related to some very basic life functions.

A review of recent NCI advances written in October by NCI staffer Steve Weiss documents several new research

areas which may dovetail with the oncogene work. A platelet-derived substance, transforming growth factor (TGF), causes a rapid proliferation of connective tissue cells (similar to the action of PDGF), and has now been used successfully to speed the healing of wounds in therapeutic situations. When placed on a laboratory culture of normal cells, TGF causes a rapid and uncontrolled proliferation which is similar to the behavior of transformed cancer cells. When the TGF is removed, the cells spontaneously revert back to normal growth patterns. Since this change is reversible, this may represent evidence that cancer is not necessarily caused by changes in genes.

The leukemia characterized by proliferation of the T-cell lymphocytes and caused by the virus HTLV-I (related to the HTLV-III which is involved in causing AIDS), is a fulminant disease which is usually fatal in three months. The T-cells are stimulated to proliferate by T-cell growth factor, and have T-cell growth factor (TCGF) receptors on their surfaces. Normal resting state cells do not have these receptors, but abnormal leukemic cells have thousands per cell. Dr. Thomas Waldman, head of the Metabolism Branch of the NCI, has developed an antibody to the TCGF receptor which prevents most of the TCGF from stimulating the cells to grow. A patient who was given this treatment has lived 12 months so far.

The use of monoclonal antibodies, antibodies grown in bacteria using gene splicing, has been extended to administering them directly into the lymph system to target cancer metastases, which frequently spread through the lymph ducts to distant lymph nodes. Dr. John Weinstein at the Laboratory of Mathematical Biology of the NCI has shown the potential to use radioactive tracer-associated antibodies to track distant metastases, and is studying the use of antibody-toxin combinations for treating such metastases.

In a related development, Dr. Jeffrey Schlom at the NCI Laboratory of Tumor Immunology and Biology developed an antibody which reacts with a protein which is found in 85% of human colon cancers and 50% of breast cancers. The antibody, called B72.3, has not reacted to any normal tissues so far tested. Dr. Schlom showed that this antibody, combined with radioactive tracers, can successfully identify tumors in mice, and is now testing this system in humans.

The mode of cancer invasion is another possible site for therapeutic intervention. Dr. Lance Liotta of the NCI Laboratory of Pathology focussed on a key protein, laminin, which is present in normal connective tissue and blood-vessel walls, which cancer cells bind to in order to penetrate through these tissues in the process of local invasion and distant metastatic spread. Dr. Liotta isolated the cancer cell surface-binding site for attaching to this protein. He then made fragments of the laminin molecule and injected them into animals with cancer; these fragments bound to the cancer cells and prevented them from binding to the animal's laminin, stopping the process of metastasis.