Fetal Antigens: A Biochemical Assay for Cancer?

Perhaps the most crucial deficiency of modern cancer therapy is the difficulty of detecting new tumors at a stage of their development when therapy has a high chance of success. Largely as a result of delayed detection, cancer can now be cured in only one of every three afflicted individuals (where "cure" is defined as survival for 5 years). This represents an increase of nearly one-third as compared to the cure rate 15 years ago, but some scientists, such as Sidney L. Arje of the American Cancer Society in New York City, suggest that as many as 90 percent of individuals with cancer could be cured with current techniques if tumors could be detected earlier.

A major problem, of course, is that many people receive medical care only irregularly, and there is thus little opportunity for physicians even to attempt detection. By the time these individuals perceive overt symptoms of a tumor, it has generally metastasized (disseminated malignant cells to other sites in the body) and a cure is unlikely. But even when medical attention is available, the most commonly used techniques—such as x-rays and palpation of the breasts and prostate—require that a tumor be moderately large to be discerned, and there is a substantial probability of metastasis.

The principal exception to this rule is the Papanicolaou stain (Pap smear), in which cells sloughed from the lining of the uterus are examined for abnormalities indicative of cancer. Increasing use of the Pap smear has lowered the incidence of fatalities from uterine cancer by 38 percent in 15 years, although only about half of all women in the United States are tested at regular intervals. But the Pap smear represents a unique case in which the sloughed off cells are readily accessible, and it is unlikely that comparable cytologic assays will be developed for cancers of other internal organs.

It would thus be extremely useful to have biochemical tests that would indicate the presence of a tumor. There are already some enzyme assays that assist in the diagnosis of cancer, points out Oscar Bodansky of the Sloan-Kettering Institute for Cancer Research, New York City. The activity of alkaline phosphatase in the blood, for example, is increased in individuals with skeletal or liver tumors; the activity of acid phosphatase is increased in individuals with prostate tumors. Most assays, though, are less specific for organ site. The activity of glutamate-oxaloacetate aminotransferase, for instance, is increased somewhat in nearly every type of cancer, as is the activity of glucose-phosphate isomerase.

These increased activities, which reflect the altered metabolism of tumors, are typically on the order of 10 to 80 percent. Since similar increases are manifested in many other diseases, these assays are generally not suitable for routine cancer screening. What is required are assays for materials unique to tumors. There has been some progress, particularly with respect to two substances called carcinoembryonic antigen and α-fetoprotein; but there is as yet no biochemical assay for cancer, and there is no prospect for the immediate development and implementation of such an assay. One by-product of research in this area, however, has been a potentially substantive improvement in techniques for assessing the results of cancer therapy.

Isozymes and Glycoproteins

The search for a biochemical cancer assay has focused primarily on a group of substances known as fetal antigens. These are proteins, glycoproteins, and polysaccharides found primarily in embryonic tissues and fetuses; they are somewhat loosely classified as antigens because of the immune response they provoke in laboratory animals (and, in some cases, in mature animals of the same species). Fetal metabolism is understood only very poorly, but it is clear that fetal tissues contain a large number of enzymes homologous to those in adult tissues. These isoenzymes or isozymes perform the same biological functions as those in adult tissues, but have different amino acid compositions, may have different substrate specificities, are generally subject to different cellular controls, and are immunologically distinct. Fetal cells also contain structural components, particularly glycoproteins, different from those in mature tissues.

It is not clear what advantage the special enzymes provide to embryonic cells, but it seems likely that they are better adapted for the more rapid, sustained growth characteristic of embryos. It is also possible that the fetal structural components play a key role in the embryo's survival in the hostile environment created by its mother's immune system. (Implantation of a fertilized egg in the mother's uterus elicits much the same immune response as transplantation of an organ, and the fetus survives only by actively interfering with that response.) Once the embryo reaches a certain stage of development, the genes that code for synthesis of the fetal components are in some fashion deactivated and become part of the cell's large library of dormant genes.

The mammalian genome contains more than 4 billion nucleotide pairs, but only a small fraction of the genes are expressed (transcribed) at any one time. Eric Davidson of the University of Southern California, Los Angeles, has shown (by molecular hybridization of cellular RNA with DNA from the genome) that the fraction expressed varies from less than 1 percent in some mature cells to as much as 12 percent in mouse embryos. Since more than 90 percent of the genome must thus normally be kept repressed, there is a great potential for inappropriate expression of genes, particularly if there is interference with the (unknown) chemical regulators of gene expression by carcinogenic chemicals or viruses. Hence it is quite possible that the expression of fetal genes in a mature cell is one cause of cancer.

In this view, espoused by investigators such as Clement L. Markert of Yale University, New Haven, Connecticut, there are no unique properties of cancer cells. Rather, there is only an aggregation of properties not normally found together in mature cells. Two of the three principal characteristics of malignancy—sustained cell division and cell migration—are also characteristic of embryonic cells. The third characteristic is a reversion of structure and metabolic activity to a more primitive or embryonic state. It is thus reasonable, Markert suggests, that cancer simply represents the activity of normal genes functioning in

abnormal patterns. This view has only recently begun to be accepted in the United States, where there has been a strong bias toward a viral origin of cancer; it has been much more widely accepted in other countries.

The similarities between malignant and embryonic cells have spurred many investigators to search for common enzymes and structural components which might provide both a way to detect tumors and a means to mount an immunological attack. But this search has been largely a hit-and-miss process, argues Norman G. Anderson, director of the Molecular Anatomy Program at Oak Ridge National Laboratory, Oak Ridge, Tennessee. What is most urgently needed, he argues, is an organized search for fetal antigens, similar to those in use for chemical carcinogens and chemotherapeutic agents. Anderson and his associates at Oak Ridge have developed an immunochromatography system to concentrate and isolate the fetal antigens that presumably are present in blood serum, urine, tissue culture supernatants, and tumor extracts. Anderson's system is new and has been little used, but meanwhile the study of fetal antigens has been provided some initial momentum by other investigators.

Among the key developments in this research were the discovery of carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). CEA is a glycoprotein that was first isolated from colon tumors in 1965 by Phil Gold and Samuel O. Freedman of the McGill University School of Medicine, Montreal. AFP, a protein that has long been known to occur in the blood of embryos and infants, was first isolated from liver tumors in 1963 by G. I. Abelov of the N. F. Gameleya Institute for Epidemiology and Microbiology, Moscow. Subsequent investigations have been carried out by many scientists, including Norman Zamcheck of Harvard Medical School, Boston, Massachusetts; Paul Lo Gerfo of the Columbia University College of Physicians and Surgeons, New York City; Thomas A. Waldmann and Richard H. Adamson of the National Cancer Institute, Bethesda, Maryland; Hans Hansen of Hoffman-La Roche Inc., Nutley, New Jersey; and E. Douglas Holyoke and T. Ming Chu of the Roswell Park Memorial Institute, Buffalo, New York.

The normal functions of CEA and AFP in embryonic tissues are still unknown, but both are relatively abundant. At about the twelfth week of gestation, for example, AFP occurs in the blood of a fetus at a concentration of about 1 million nanograms per milliliter. By birth, the concentration has dropped to about 30,000 ng/ml, and in adults it is usually less than 30 ng/ml.

Table 1. Typical results from studies of CEA concentrations in patients with various diseases. [Source: U.S. Food and Drug Administration]

	Percentage of patients showing CEA at concentrations (ng/ml):		
	0–2.5	2.6–10	>10
Healthy subjects			
Nonsmokers	97	3	0
Smokers	81	18	1
Carcinomas			
Colorectal	28	37	35
Pulmonary	24	50	26
Pancreatic	9	56	35
Breast	53	33	14
Others	51	40	9
Nonmalignant diseases			
Rectal polyps	81	18	1
Alcoholic cirrhosis	29	69	2
Ulcerative colitis	69	26	5
Emphysema	43	53	4

Both CEA and AFP were initially thought to be associated only with fetal tissues and with the specific tumors from which they were first isolated. Many studies have now shown, however, that elevated concentrations of both CEA and AFP are found in the blood of patients with several other types of tumors. More important, these studies have shown that elevated concentrations are not found in all patients with a given type of tumor, and are sometimes found in patients with certain types of nonmalignant disease.

The greatest amount of information is available about CEA, which has been studied in more than 10,000 subjects at some 100 institutions in the United States, Canada, and England. These studies were conducted under the auspices of Hoffman-La Roche as part of its efforts to document the reliability of a radioimmunoassay kit that can be used by practicing physicians to measure CEA concentrations.

In most patients with tumors, concentrations of CEA were higher than 2.5 ng/ml. Concentrations were elevated in 72 percent of patients with colon and rectal tumors, in 76 percent of patients with lung tumors, and in 91 percent of patients with tumors of the pancreas. But concentrations were also elevated in 19 percent of smokers, 57 percent of patients with emphysema, and 71 percent of patients with alcoholic cirrhosis of the liver. Other examples of results from the survey are shown in Table 1. The smaller number of data available for AFP are similar to those for CEA, although the assay is specific for different tumors; most "false positive" measurements of AFP occur in patients with liver diseases such as hepatitis.

Assays for the two antigens are thus not suitable biochemical tests for cancer, since low concentrations are not proof that a tumor is not there and high concentrations are not proof that it is. The assays are, however, useful as an adjunct to diagnosis by other methods. More important, they are useful in assessing the course of therapy.

Therapy of internal tumors is often a haphazard process since it is generally difficult to ascertain when a tumor has completely disappeared. Chemotherapy, for example, might thus be discontinued too soon, allowing reemergence of the tumor, or continued for too long, exposing the patient to unnecessary side effects. If a tumor is surgically excised, it is often difficult to know whether all the malignant cells have been removed or whether there have been undiscerned metastases that require further treatment. The antigen assays provide a way to circumvent these problems, and it is for this primary use that the Hoffman-La Roche assay kit was licensed by the U.S. Food and Drug Administration this year.

Studies by several investigators, especially Zamcheck, Holyoke, Chu, and Waldmann, have shown that when an antigen-producing tumor is surgically removed, the concentration of antigen generally falls to normal levels. Failure of the antigen concentration to decrease or a subsequent increase in the concentration are generally signs that not all of the tumor has been removed and that additional treatment is required. Frequently these recurrent elevated concentrations occur before the new tumor is clinically detectable; they thus offer the potential for therapy while the new tumor is still small.

In sum, the immediate future looks bright for use of the antigens in assessing therapy, but less bright for their use as a cancer screen. The Hoffman-La Roche studies required more than 4 years to produce a license for limited use of a CEA assay, and it is safe to assume that an antigen for screening would require even more studies. Since there is not now even a candidate antigen for screening, it will apparently be many years before a biochemical assay for cancer will be in use.—THOMAS H. MAUGH II