Biochemistry of Cancer Cells: Focus on the Cell Surface

There is no doubt that cancer cells differ from their normal cell counterparts. What scientists want to identify is the biochemical change or changes that produce their altered properties and behavior. The problem is important because identification of such a change may mean that it can be prevented or reversed and malignancy cured. The solution, however, is also elusive, even though it has been the target of considerable scientific enterprise, much of which, during the last 5 years, has been directed at changes in cell membranes following transformation—the conversion of normal cells to cancer cells.

Earlier research frequently centered on the Warburg effect, named after its discoverer, Otto Warburg. Fifty years ago, Warburg observed that tumor cells had a higher rate of glycolysis than did normal cells. Glycolysis is a series of reactions, not requiring oxygen, by which glucose is partially broken down and some of its energy thus recovered for the cell's use. This pathway is an inefficient energy source compared to the aerobic processes that produce most of the energy in normal mammalian cells. Warburg thought that cancer cells originate from normal cells as a result of irreversible damage to their aerobic energy-producing systems and a shift to glycolysis. But the relation between the Warburg effect and transformation is still unclear.

Tumor Cells and Glycolysis

Since not all tumor cells have increased glycolysis, it does not appear to be a prerequisite of the malignant state. This problem—determining whether a particular difference is a cause or effect of the altered properties of transformed cells—plagues investigators even today. Moreover, Warburg's theory does not account for the aberrant properties of tumor cells. Of these, the capacity to metastasize and the loss of growth control are the most characteristic.

Metastasis is the escape of tumor cells, which appear to be less "sticky" than their normal counterparts, from the tumor mass and their migration to other parts of the body where they may form new tumors. Loss of growth control means that cancer cells continue to divide even under conditions in which normal cells divide only to replace dead cells. This behavior is reflected in vitro by a phenomenon called density-dependent inhibition of growth. In culture, most normal cells stop dividing when they become crowded together; they form a single layer of cells. Transformed cells, on the other hand, continue to divide, piling up layer upon layer of cells.

Because the cell surface membrane is the structure through which cells communicate and interact, both with each other and with their environment, many investigators think that membrane alterations could cause the decreased cellular adhesiveness and loss of growth control seen in tumor cells. Such membrane alterations are thought to be the result of changes in the cell genome which may be induced by radiation, or by carcinogenic chemicals or viruses or which may arise "spontaneously."

The complexity of mammalian cells is another problem confronting investigators who want to study their biochemistry. Because of it, much remains to be learned about mammalian cell structure and function, especially about the control mechanisms that regulate gene expression and all cell activities. There is, however, a growing appreciation that membranes, once thought to be relatively inert cell coverings, are dynamic structures that participate in the regulatory mechanisms. The precise role of membranes, in normal cells and especially in transformation, is unclear. Investigators, employing a number of strategies, have detected significant membrane alterations in transformed cells. A unifying concept that explains the membrane's role in transformation has not yet evolved from the different lines of investigation. Instead, there is considerable dispute as to what will prove most pertinent to the cancer problem.

Membrane changes in transformed cells can be studied directly by biochemical analysis of important membrane components such as glycoproteins and glycolipids or indirectly by using a group of plant proteins called lectins to probe the cell surface. This latter strategy has been employed by a number of investigators, including Garth Nicolson of the Salk Institute, San Diego, California; Max Burger, first at Princeton University, Princeton, New Jersey, and more recently at the University of Basel, Switzerland; and Leo Sachs, at the Weizmann Institute of Science, Rehovot, Israel. They found that lectins can be used to detect membrane differences between normal and transformed cells. Transformed cells agglutinate in the presence of lectins while the corresponding normal cells, under the same conditions, do not. Agglutination occurs because lectin molecules, which have several binding sites, bind to cell surface receptors—probably the carbohydrate portion of membrane glycoproteins—on two or more cells and thus cause them to clump.

Mobility of Lectin Receptors

Nicolson thinks that the greater agglutinability of transformed cells is due to higher mobility of the lectin receptors in cancer cell membranes than in normal cell membranes. With S. J. Singer of the University of California at San Diego, he proposed the "fluid mosaic model" for the structure of cell membranes. According to this model, the surface membranes of cells are dynamic fluid structures in which membrane components may migrate laterally in the membrane plane.

Using the lectin concanavalin A (Con A) labeled by conjugation to the iron-containing protein ferritin, Nicolson found that the Con A binding sites on the surface of mouse fibroblasts (3T3 cells) transformed by the oncogenic virus SV40 formed clusters more easily than did those on normal 3T3 cells. In some preparations of aggregated transformed cells, he could see clusters of ferritin-labeled Con A at the sites of cell contact.

Nicolson thinks that cluster formation requires migration of the receptors; when transformed cells bind lectins, migration of the receptors allows the polyvalent lectins to contact other receptors, form additional linkages, and thus produce clusters of receptors and lectin molecules. This increases local densities of bound lectin molecules so

that multiple bridges can form between adjacent cells. Receptors in the membranes of normal cells migrate less readily and do not form clusters well enough for agglutination to occur.

Alterations in the composition of the membrane or in the structure of the lectin receptors following transformation are potential explanations for increased receptor mobility. Nicolson, however, favors the suggestion (made by a number of investigators) that submembrane structures such as microtubules and microfilaments help to regulate the movement of membrane constituents. Drugs that disrupt microtubules or microfilaments alter lectin-mediated agglutination of some cells. Microtubules form the mitotic spindle and are essential for cell locomotion and division (*Science*, 28 September 1973). These results imply a possible connection between components of the cell membrane and structures in the cytoplasm.

Membrane Fluidity and Differentiation

Using a different cell system, Sachs and Michael Inbar of the Weizmann Institute demonstrated a relationship between membrane fluidity and the capacity of cells to differentiate. They employed cultured myeloblastic cells obtained from mice with myeloid leukemia. These are immature cells that have not differentiated to such normal mature forms as macrophages and granulocytes. Sachs and Inbar had previously shown that a protein released from various cells induced certain undifferentiated cells to differentiate. Some of the cultured leukemia cells differentiate in the presence of the protein; others do not.

Sachs and Inbar probed the membrane differences between the two cell types with Con A labeled with a fluorescent tag. They found that the fluorescent Con A molecules formed caps or clusters at one pole on 50 percent of cells that differentiate but on only 5 percent of cells that do not. This indicates that Con A receptors are more mobile in the first cell type. Sachs and Inbar also found that Con A receptor sites were more mobile in normal lymphocytes than in malignant lymphoma cells. This result is apparently the opposite of that for normal and transformed 3T3 fibroblasts. However, mature white blood cells differ markedly from fibroblasts.

The molecular basis of the enhanced lectin agglutinability of transformed cells is as yet unknown, but normal cells can be altered so that they behave more like transformed cells. Max Burger and his colleagues found that mild treatment of normal cells with proteases (enzymes that break down proteins) made them as susceptible as transformed cells to agglutination by lectins. The proteases also stimulated a round of cell division, temporarily releasing the cells from the normal density-dependent inhibition of growth. Burger hypothesizes that derangements in protease activity, especially on membrane components, may be implicated in the development of the characteristics of transformation. The observation by Burger and Hans Schnebli of the University of Basel that protease inhibitors also inhibit the growth of transformed mouse and hamster cells tends to support this hypothesis.

Burger has suggested that transformation produces a conformational rearrangement of the cell surface which exposes hidden receptors and allows them to bind lectin molecules. Transformed cells could then bind more lectin molecules, and, since there would be more opportunities for lectin bridges to form between cells, agglutination would be enhanced. Unlike most other investigators, who have not found such increases, he finds that transformed cells bind three to five times as much lectin as do normal cells. Burger hypothesizes that site exposure results from protease activity.

A variety of changes in membrane structure and function occur in cyclical fashion during the cell cycle. According to Burger, these include an altered response to lectins. He found that normal dividing cells, in contrast to normal nondividing cells, were agglutinated by wheat germ agglutinin. They also bound more lectin molecules than did controls. These and other observations have led Burger to speculate that transformation so alters the cell membrane that it can no longer undergo the normal cyclical changes that terminate cell division—in other words, the cell is effectively trapped in the dividing state.

Increased agglutinability by lectins is not invariably associated with the transformed state. For example, George Poste of Roswell Park Memorial Institute, Buffalo, New York, has shown that infection of normal cells by a number of viruses that are not oncogenic increases the cells' susceptibility to agglutination. The preponderance of evidence, however, does indicate an association between transformation and increased lectin agglutinability although the mechanism producing enhanced agglutinability is still disputed. Burger favors the hypothesis that increased availability of lectin receptors in transformed cells is responsible while Nicolson (among others) thinks that increased receptor mobility in such cells is the most important factor.

Analysis of the chemical composition of cells and their membranes is another strategy used by researchers attempting to pin down the differences between normal and malignant cells. Of particular interest are the glycoproteins and glycolipids located in the cell membrane and the enzymes required for their synthesis and breakdown. Glycoproteins and glycolipids consist of a carbohydrate portion plus protein or lipid. The carbohydrates apparently project from the outer membrane surface, where they may be involved in interactions between cells and could be the moieties involved in cell contact and density-dependent inhibition of growth. Lectin receptors are probably glycoproteins.

Glycoproteins and Glycolipids

A number of investigators have found that transformation can alter the composition of membrane glycoproteins and glycolipids. Leonard Warren, Mary Glick, and Clayton Buck of the University of Pennsylvania Medical School, Philadelphia, analyzed glycopeptides removed from cell membrane glycoproteins by the protease trypsin. One fraction of high molecular weight glycopeptides greatly increased in cells transformed "spontaneously" or by DNA or RNA viruses. The increase appeared to be correlated with transformation, and was not found in cells infected by a nontransforming virus. The increase also occurred in chick embryo fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus (RSV) only at the permissive temperature but not at the nonpermissive temperature.

Temperature-sensitive mutants such as this RSV mutant (RSV is an oncogenic RNA virus) are proving to be valuable tools for studying transformation. They transform cells at one temperature, the permissive temperature, but not at higher or nonpermissive temperatures. Transformation can thus be accomplished at will, simply by adjusting the temperature. The change is

reversible, and virus production is maintained at both temperatures. This eliminates the possibility that virus infection rather than transformation produces the effects observed.

According to Warren, Buck, and J. P. Fuhrer, also of the University of Pennsylvania Medical School, the large glycopeptides are formed by addition of sialic acid to precursor glycopeptides. The transfer is catalyzed by an enzyme, a sialyl transferase, whose presence correlates with that of the large glycopeptide fraction.

The function in transformed cells of the membrane glycoproteins from which the glycopeptide fraction is derived is not known. The presence of the glycopeptide fraction depends on cell division, since this fraction is not found in transformed cells that are not dividing. Glick and Buck found a glycopeptide pattern in normal dividing cells similar to that in transformed cells. If the glycopeptides of the two kinds of cells are indeed the same, this would be another resemblance between malignant and dividing cells. Warren said, however, that the large glycopeptides had no measurable affinity for Con A so they are unlikely to be the lectin receptor and thus would not account for the enhanced agglutinability observed by Burger in dividing cells.

Transformation of cells by a chemical carcinogen did not have the same effect on glycopeptides as did viral transformation. Glick, collaborating with Sachs at the Weizmann Institute, observed no differences in the glycopeptide patterns of chemically transformed cells in culture. Cells from tumors that developed in vivo from the transformed cells did have an altered glycopeptide pattern similar to that of virally transformed cultured cells. Moreover, the extent of the change correlated with the cells' tumorigenicity.

Glycolipid Pattern Simplified

Alterations in glycolipid patterns of transformed cells follow a general trend toward simplification; the more complex glycolipids decline in concentration or disappear after transformation. Roscoe Brady and his colleagues at the National Institute of Neurological Disease and Stroke, Bethesda, Maryland, found that certain gangliosides (complex sialic acid–containing glycolipids) disappeared following transformation of mouse cells by DNA viruses. Synthesis of these glycolipids requires sequential addition of sugars and sugar derivatives to the growing molecules. Virally transformed cells lack an enzyme necessary for one of the steps in glycolipid synthesis. Brady did not find these changes in a spontaneously transformed cell line capable of producing tumors in vivo.

Transformation by RNA viruses like RSV also alters the glycolipid pattern of membranes, according to Sen-itiroh Hakomori and his associates at the University of Washington School of Public Health and Community Medicine, Seattle. The concentrations of certain complex glycolipids decreased in transformed chick fibroblasts. The same glycolipids increased when normal cells in culture stopped dividing because of density. Hakomori hypothesizes that these glycolipids form contact-sensitive groups in the cell membrane which control division. By losing the capacity to synthesize the proper glycolipids at contact, transformed cells escape from density-dependent inhibition of growth. Since some investigators have not found glycolipid changes in transformed cells, Hakomori has suggested that glycoproteins may serve as the contact-sensitive groups in some cell types.

Analysis of cell glycolipids is frequently performed on extracts of whole cells, rather than of membrane preparations. The conclusion that glycolipids are involved in surface phenomena therefore depends on the validity of the assumption that they are located only in the outer membranes. Additional evidence for their involvement has been provided by Hakomori. When he added globoside, a complex glycolipid, to the culture medium of transformed cells it was incorporated into the surface membrane. Moreover, the growth rate of the cells slowed because initiation of DNA synthesis, a prerequisite for mitosis, was delayed.

Another advantage of temperature-sensitive mutants is that they permit identification of very early changes due to transformation—changes that may be causes rather than effects of the altered characteristics of transformed cells. Transformation of cells infected with the mutant begins as soon as they are transferred to the permissive temperature, and they can be studied at short intervals following transfer. Phillips Robbins and Gary Wickus at the Massachusetts Institute of Technology, Cambridge, found an altered protein pattern in the membranes of transformed cells that may be one of the earliest changes of transformation.

According to Robbins and Wickus, a membrane protein with a molecular weight of 45,000 was reduced in amount in chick embryo fibroblasts infected with the temperature-sensitive mutant of RSV and maintained at the permissive temperature but not in cells maintained at the nonpermissive temperature. The reduction was associated with transformation rather than with the temperature change; it was found at both temperatures in cells transformed by wild type RSV and at neither temperature in uninfected cells.

The concentration of this protein did not decrease until 3 to 6 hours after transformation was initiated. However, Robbins and Wickus observed a reduction in the concentration of another protein, of as yet uncertain location. This change, also associated with transformation, occurred within 3 hours after transformation was initiated, and may be one of the first effects of transformation.

Enzyme Changes in Transformed Cells

Other investigators have focused their attention on changes in the enzymes produced by transformed cells. Edward Reich and his colleagues at the Rockefeller University, New York City, demonstrated that a number of cultured avian or mammalian cell lines, transformed by either DNA or RNA viruses, produced an enzyme that breaks down fibrin (the protein that forms blood clots) when the incubation media were supplemented with appropriate blood serums. Generation of the fibrinolytic activity required the interaction of two proteins. One is present in normal serums; the other is produced and released into the incubation medium by transformed cells, but not by normal ones.

Reich identified the cell factor as a protease and the serum factor as plasminogen. Plasminogen is the inactive precursor of plasmin, an enzyme that breaks down fibrin. Formation of plasmin normally requires the partial breakdown of plasminogen by a protease; in this case, the protease is secreted by transformed cells.

According to Reich, production of the protease is consistently associated with transformation. When chick embryo fibroblasts were infected with a temperature-sensitive mutant of RSV and cooled to the permissive temperature, appearance of the fibrinolytic activity preceded the morphological evi-

dences of transformation by 4 to 8 hours. When the cells were warmed to the nonpermissive temperature, the fibrinolytic activity declined, which indicated that enzyme production ceased. Alterations of cell morphology resulting from transformation appear to require the action of plasmin. Removal of plasminogen from the incubating serums prevented expression of the alterations.

The role of plasmin in cell transformation is as yet unknown, and Reich prefers not to speculate at this time. However, Burger has implicated proteases in transformation. And Robbins has hypothesized that protease activity could account for the protein loss he saw during transformation. Nicolson considers Reich's contribution to be significant because it may help explain how cancers metastasize or spread. A protease could enable malignant cells to invade other tissues by breaking down the intracellular matrix or cement that binds cells together. The effect of plasmin on the cell surface membrane will no doubt receive intense scrutiny.

Regulatory Signals

When investigators were looking for a way in which signals regulating cell division might be transmitted from membrane to nucleus or from nucleus to membrane, they turned their attention to that ubiquitous regulator adenosine 3′,5′-monophosphate (cyclic AMP) and more recently to guanosine 3′,5′-monophosphate (cyclic GMP). Stimulation of cell division is usually associated with low cyclic AMP and high cyclic GMP concentrations or with attainment of the proper ratio of the two nucleotides (*Science*, 12 October 1973). For example, when Burger treated normal cells with proteases and stimulated them to divide, the cyclic AMP concentration declined. Addition of a cyclic AMP derivative during the protease treatment prevented the growth response.

In order to determine what causes the decreases in cyclic AMP after transformation, Ira Pastan and his colleagues at the National Cancer Institute (NCI), Bethesda, Maryland, measured the activity of adenylate cyclase, the enzyme that catalyzes cyclic AMP synthesis, in cells transformed by RSV. They found that its activity declined after transformation. When they infected the cells with temperature-sensitive mutants of RSV and cooled the cells to the permissive temperature, adenylate cyclase activity and the cyclic AMP concentration decreased during transformation. Since adenylate cyclase is a membrane enzyme, Pastan hypothesizes that alterations in membrane glycolipids or glycoproteins caused by transformation affect the enzyme activity.

Cyclic AMP may interact with microtubules and microfilaments, as proposed by a number of investigators. One of them, Theodore Puck at the University of Colorado Medical Center, Denver, has shown that a cyclic AMP derivative converts Chinese hamster ovary cells from a morphological form that resembles transformed cells to a form more like that of normal fibroblasts. Drugs that disrupt microtubules and microfilaments prevented the action of the cyclic AMP derivative. Puck postulates that cyclic AMP affects organization of microtubules and microfilaments.

Whatever the role of cyclic AMP in the mechanism of transformation, there are indications that it can arrest tumor growth in vivo. Pietro Gullino and his colleague Yoon Sang Cho-Chung at NCI, administered dibutyryl cyclic AMP (an analog of cyclic AMP) to rats with mammary tumors. The tumors stopped growing during the 3 weeks of treatment, and growth resumed when the treatment ceased. Gullino believes that dibutyryl cyclic AMP produced this effect by increasing breakdown of the tumor cells. When the drug was given, the activity of acid ribonuclease, a lysosomal enzyme that breaks down RNA, increased twofold. Gullino had previously shown that regression of certain tumors is accompanied by sharp increases in the activity of six lysosomal enzymes, including acid ribonuclease.

Not all investigators think that cell contact is a significant factor in regulating cell division. Robert Holley of the Salk Institute suggests that growth control of mammalian cells involves interaction among many factors including a number of regulatory agents present in blood serum. He bases his theory partly on his observations that growth of cultured cells depends on the serum concentration of the incubating medium. Normal cells stop dividing at higher densities as the serum concentration increases. Transformed cells, on the other hand, are less dependent on the serum concentration of the medium. They continue to divide even when deprived of serum. Holley has identified four serum factors required for initiating DNA synthesis in normal cells; he is now trying to isolate and characterize them.

In Holley's view, normal cells divide when the intracellular concentration of the necessary nutrients is adequate; they need no additional signals. The availability of these nutrients would be controlled by the serum factors that influence permeability of the cell membrane. Cancer occurs when changes in the membrane release the cells from regulation by the serum factors. Nutrients enter freely, and cell division is continuous. The membrane thus plays a crucial role in oncogenesis, according to Holley's hypothesis, but it is a different role than that proposed by others.

Membrane Changes and Transformation

Investigators have found a number of membrane changes associated with transformation; changes in lectin agglutinability, in glycolipid and glycoprotein composition, in proteins, and in enzymes. At present, no one really knows how these observations are related—if they are related. Alterations in membrane composition could affect the fluidity of the membrane or the availability of receptors, or both. So could activity of a protease. Cyclic AMP and GMP may also be involved in the events that regulate cell division. All of this emphasizes the enormous complexity of the mammalian cell and of the problems that must be solved to gain a clearer understanding of the biochemistry of cancer cells.

Most investigators stressed that a better understanding of the biochemistry of normal cells, including the mechanisms that regulate gene expression is also required. Since their evidence indicates that altered gene expression in transformed cells may be reflected in changes at the cell surface, the relation between genes, membranes, and malignancy provides a promising area for exploration.—JEAN L. MARX