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GROWTH FACTORS, ONCOGENES AND THE AUTOCRINE HYPOTHESIS

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CANCER can be defined as the uncontrolled overgrowth of cells. Research shows that malignant cells can use normal cellular processes that govern cell division and growth. The study of the growth of normal cells has led to the discovery of polypeptide hormones required by the cells for growth, or growth factors (1). Growth factors bind specific cellular receptors and stimulate the intracellular events that ultimately lead to cell division. Cellular proliferation and differentiation are tightly regulated by cellular genes. Alteration of these genes by mutation or chromosomal damage can result in the loss of growth regulation, producing malignancy (2).

The study of the causes of malignant growth has focused on the molecular biology of deoxyribonucleic acid (DNA) and cancer-causing genes, or oncogenes. The discovery that oncogenes are related to the genes that control normal growth of cells, or proto-oncogenes, has shown that both normal and malignant patterns of growth involve similar growth mechanisms (2, 3). Oncogenes and growth factors may act synergistically in malignant transformation (4). Some oncogenes can render cells independent of growth factors by enabling a cell to produce and respond to its own growth factor. This process of autostimulatory growth is known as autocrine growth (5). Oncogenes, then, cause cancer by using established pathways of normal growth of cells.

METHODS OF STUDY

Cell culture has become a useful technique for studying growth and transformation in vitro. Transformation is the conversion to a state of unrestrained growth in culture, resembling, or identical to, the growth of tumor in vivo. Serum is necessary for cell growth in culture (6). It contains such growth factors as platelet derived growth factor (PDGF) (7), epidermal growth factor (EGF) (1), insulin and insulin-like growth factor (IGF) (8). Normal growth of cells is inhibited by high cell density and requires anchorage of the cells to the agar medium (9). Transformed cells (tumor cells), however, show less stringent requirements. They require little or no exogenous growth factors; they lose density inhibition and exhibit anchorage-independent growth. These properties in vitro correlate closely with the ability to cause tumors in vivo (10). Thus, a model for in vitro study of the growth of tumors can be analyzed and modified.

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GROWTH FACTORS

Growth factors are substances that enable cells to divide and grow. Their actions are mediated by the binding of the factor to specific, high affinity receptors on the surface of the cell. This binding, in turn, initiates the intracellular cascade of events that eventually lead to cell division. Insulin, somatomedins, growth hormone, interleukins, EGF and PDGF are some of the growth factors that have been identified and described. Growth factors may act in an endocrine, paracrine or autocrine manner (5). Endocrine hormones are secreted by glands and carried by the bloodstream. They affect target organs far from the site of origin. Insulin, for example, is secreted by the pancreas and has an effect in the liver, causing it to store glucose. Paracrine secretion has its effect by local diffusion of the hormone to neighboring cells of a different type. PDGF from platelets stimulates proliferation of vascular smooth muscle cells (11). Autocrine secretion is characterized by the ability of a cell to respond to its own secreted growth factors. Interleukin-2 (IL-2), for example, is secreted by lymphocytes that can also respond to IL-2 by dividing and multiplying (12).

Cell division appears to require two sequential signals or growth factors. The first signal renders cells competent to divide; the second allows progression into division (13). Growth factors in the first category include PDGF and interleukins. They act in a local manner and are specific for

certain cell types. Those in the second category include insulin, EGF and somatomedins, which have a broad spectrum of action and can work systemically.

Growth factors may also be classified according to their functional roles (14): 1, those involved in embryogenesis and development, such as EGF (15) or insulin-like growth factor (IGF) (8); 2, those associated with adult cells with high turnover rates as hematopoietic cells or epithelium, such as erythropoietin, IL-2 (12) or EGF (15); 3, growth factors involved in wound healing and inflammation, such as PDGF (11, 16, 17), and 4, those associated with tumor cells, such as PDGF (18), transforming growth factor alpha (TGFα) (19) or bombesin (20). Growth factors are not limited to only one of these groups—some function in all four categories. TGFα and PDGF are two of the most extensively studied growth factors; their functions serve as examples of actions by growth factors on target cells.

GROWTH FACTORS AND NORMAL DEVELOPMENT

PDGF has multiple functions in normal cells. PDGF is a polypeptide that is stored in platelet granules and released when blood clots. It is the major growth factor in serum for fibroblasts, smooth muscle cells and other cells derived from connective tissue (21). Binding of PDGF to its receptor initiates a cascade of intracellular events, such as tyrosine phosphorylation, stimulation of amino acid transport, appearance of rare cytoplasmic proteins and, ultimately, the capacity to divide in response to other growth factors, such as insulin or EGF (7, 22). PDGF is involved in embryogenesis, cellular development and repair of cellular injuries. In the embryo, PDGF and PDGF receptors are found in placental cytotrophoblasts; their growth appears to be driven by an autostimulatory (autocrine) loop (21). Cellular development in aortic smooth muscle of rats appears to be dependent on PDGF; PDGF is found in the smooth muscle of young, developing rats, but not in adult rats (23). Repair of injury to blood vessels also requires PDGF. It is produced by endothelial cells and macrophages at the site of vascular injury. PDGF stimulates proliferation of vascular smooth muscle cells by way of a paracrine mechanism (11, 16, 17). In summary, PDGF is important in the regulation of embryogenesis, cellular development and repair of injury.

In 1983, it was found that PDGF is virtually identical to the transforming protein of a carcinoma-causing virus, the simian sarcoma virus (SSV) (24, 25). This discovery provided direct evidence for the relation of neoplastic transformation to normal cellular growth mechanisms. In addition to its actions in normal cell growth, PDGF can also function as a transforming growth factor.

TRANSFORMING GROWTH FACTORS

In addition to the discovery of growth factors that are active in normal growth and differentiation, growth-promoting transforming polypeptides, or transforming growth factors, have also been discovered. These factors produce the transformed phenotype in untransformed cells. Functionally, they act as strong mitogens, cause loss of density inhibition of growth and allow anchorage-independent growth (19).

TGFα was one of the first of these factors to be described. In mice, it is released by cells infected with a sarcoma-causing virus (26). In humans, it is also found in multiple tumor cell lines, including sarcoma (26), carcinoma of the breast (27), neuroblastoma (28), melanoma (29) and carcinoma of the colon (30). TGFα is also synthesized during early fetal development (31). This suggests that TGFα is normally expressed in the fetus, and that its abnormal expression in fully differentiated cells may lead to transformation.

TGFα is antigenically distinct from EGF but is capable of binding the EGF receptor; indeed, its actions are mediated by EGF receptors (32). The actions of TGFα and all growth factors are a function not only of the growth factor itself, but also of the receptors present in the cell at a given time. TGFα and PDGF are growth factors necessary for normal growth and development; both can also cause malignant transformation, under certain conditions. These conditions are sometimes affected by oncogenes.

ONCOGENES

An oncogene contains genetic material that transforms cells and produces cancer. Experiments on the transfer of genes were the first to demonstrate that cancer is controlled by genes. DNA from murine tumor cells was isolated and introduced into normal murine cells; some of the cells acquired malignant characteristics (33). The results from such an experiment showed that the information necessary to produce cancer is present in DNA. These are the oncogenes.

Oncogenes can be found in cancer-causing viruses as well as in tumor cells. They can use established, normal growth regulatory pathways to effect malignant transformation. Normal cells

contain genes that regulate growth, differentiation and embryologic development. Analysis of DNA has shown that these regulatory genes are homologous to oncogenes; they are the proto-oncogenes, or cellular oncogenes (2).

To date, more than 20 cellular oncogenes have been identified, showing high evolutionary conservation between species. Cellular oncogenes are expressed at various times during growth and development; they are believed to be active in governing differentiation and to possess key regulatory functions (3). In their native form, they do not cause malignant transformation; however, it is postulated that cellular oncogenes may be activated, causing cancer (34). Multiple examples of activated cellular oncogenes have been found in a variety of human tumors, including neuroblastoma (35), pulmonary carcinoma (36, 37), lymphoma (38) and carcinoma of the bladder (39, 40). Oncoviruses are now known to have transduced or borrowed their oncogenes from the cellular oncogenes of their hosts (34, 41). Infection with an oncovirus may provide the cell with an activated oncogene, leading to transformation.

MECHANISMS OF ACTIVATION OF ONCOGENES

Proto-oncogenes may be activated by various mechanisms. These mechanisms either lead to excess expression of proto-oncogenes or to expression of a modified proto-oncogene. These mechanisms include gene amplification, point mutation, chromosomal translocation and viral activation.

Amplification of the *myc* oncogene has been observed in the DNA of some tumor cell lines, including neuroblastoma (35) and pulmonary carcinoma (42). Multiple copies of an oncogene (amplification) may result in overexpression. Point mutation in the *ras* oncogene has been implicated in the pathogenesis of tumors in the human bladder (39, 40) and colon (43, 44). Point mutation, a simple change of the nucleotide, causes a substitution of amino acids; this change results in the expression of a modified proto-oncogene product. Chromosomal translocation has been found in Burkitt's lymphoma cells (38). It exchanges the regulatory region of a proto-oncogene with that of an immunoglobulin gene. This exchange results in deregulation and an increase in proto-oncogene expression. Viral activation of proto-oncogenes can occur through two mechanisms. A virus can insert itself next to a proto-oncogene and activate its expression (45). Alternatively, a virus can transduce or borrow a proto-oncogene from a host and incorporate it into its own genetic material. This oncogene-carrying virus can infect other cells and cause cancer. Alteration of either regulatory or structural functions of proto-oncogenes by gene amplification, point mutation, chomosomal translocation or viral activation can result in the formation of tumors.

Oncogenes may not act alone in transformation. Malignant conversion may require two cooperating oncogenes, and carcinogenesis may be a multistep process (46, 47). In experiments done on fibroblasts of rats, two different oncogenes had no effect when each acted alone, but together acted synergistically to transform cells (46).

Similarly, some oncogenes can transform only those cells that have been previously treated with chemical carcinogens (48). Chemical mutagens and some oncogenes can immortalize cell lines; other oncogenes can transform these immortalized cells, but not normal cells (48). Substances capable of immortalization generally act in the nucleus; transforming substances act on the inner membrane of the plasma, perhaps at the level of growth factors and their receptors (46). In fact, some oncogenes render the cells independent of growth factors.

THE AUTOCRINE THEORY

One way by which a cell can become independent of a growth factor is through autocrine secretion, by simultaneously manufacturing and responding to its own growth factor (5). Much evidence exists to support the autocrine hypothesis. Various polypeptides, including PDGF, bombesin, TGFα and chicken myelomonocytic growth factor, have been shown to function by way of an autocrine loop in cells with cancer (20, 49–51). Oncogenes may encode for growth factors and their receptors, or may affect the postreceptor intracellular signal.

The most thoroughly studied model of autocrine secretion is the cell line of human small cell lung carcinoma (SCLC). These cells possess all the requirements for autocrine growth, namely, production and secretion of a growth factor, receptors for that same growth factor and inhibition of growth stimulation by specific antigrowth factor antibodies. SCLC cells secrete bombesin (gastrin-releasing peptide), which stimulates growth. SCLC cells contain high concentrations of bombesin and have high affinity bombesin receptors (52–54). Monoclonal antibombesin antibodies prevent binding of bombesin and block growth of tumor in vitro and in vivo (20). This

finding provides direct evidence for autocrine growth in such cells.

Transformation by SSV also occurs by way of an autocrine mechanism. The protein product of SSV has been shown to be nearly identical to the beta chain of PDGF (24, 25). Cells transformed by SSV secrete PDGF-like molecules that bind PDGF receptors and stimulate growth (18). This stimulation is prevented by anti-PDGF antibodies, which block receptor binding (50). Transformation by the SSV oncogene, therefore, occurs by way of a growth factor that functions in normal cell growth.

Autocrine growth has also been described in normal, untransformed cells. T cells, for example, use IL-2 as an autocrine growth factor (12). Autocrine growth is a normal mechanism by which cells can confer upon themselves a growth advantage when required. Triggered by oncogenes, malignant cells can subvert this mechanism to promote their own uninhibited growth. In addition to coding for a growth factor, such as PDGF, an oncogene may also affect the receptor or the postreceptor signal. The autocrine hypothesis can be broadened to include the importance of these pathways (55).

OTHER AUTOCRINE MECHANISMS

Growth factor effects are mediated by specific cell surface receptors. Once binding occurs, receptors are activated and a myriad of intracellular processes occur. One early event is the phosphorylation of tyrosine. In fact, EGF, PDGF and insulin receptors contain tyrosine phosphorylating (kinase) activity with the receptor molecule (56–58). Modification of protein configuration by reversible phosphorylation allows a change in the functional state of that protein. Regulation of cell function can occur by this mechanism. IGF binding also increases the transport of glucose and the synthesis of DNA, ribonucleic acid (RNA) and protein and promotes cellular proliferation and differentiation (8). PDGF binding of its receptor induces synthesis of many different proteins involved in cell division (6).

The nature of the intracellular messengers between cell surface receptors and the nucleus is not known; guanine nucleotides (guanosine triphosphate and guanosine diphosphate) and cyclic adenosine monophosphate may be some of the second messengers. The following examples show specifically how oncogenes transform cells by their effects on growth factor receptors and postreceptor signals.

ALTERATIONS AT THE LEVEL OF RECEPTORS

Oncogenes can code for, or alter expression of, growth factor receptors. For example, an oncogene codes for an incomplete EGF receptor that no longer requires ligand binding for activation (59). Another oncogene codes for a protein similar to mononuclear phagocyte colony-stimulating factor (CSF-1) receptor, but it may be permanently activated (60). A permanently activated receptor obviates the need for growth factor binding and sends a constant growth-stimulating signal to the nucleus. Some transformed cells show an increased number of EGF receptors at the cell surface (61); a normal receptor can be amplified and rearranged (62) or modified with tumor-specific antigen (63). These oncogene actions can render a cell more responsive to, or even independent of, growth factors.

MODIFICATION OF POSTRECEPTOR PATHWAYS

Oncogenes can modify the intracellular signals that occur after growth factor binding. Many oncogenes code for tyrosine kinase (64), a protein often associated with growth factor receptors and known to trigger second messengers, leading to cell division. This protein can release the cell from having to express either the growth factor or its receptor, resulting in uncontrolled cellular proliferation. Some products of oncogenes bind GTP (65), which may be involved in the postreceptor signal process. Other products of oncogenes bind DNA and directly affect the actions of DNA in the nucleus (66). Thus, oncogenes can alter the normal growth regulatory pathways in a number of different places, thereby producing cancer.

SUMMARY

Many aspects must be studied when considering theories of oncogenesis. Growth factors, the polypeptide hormones that are necessary for cell growth, and oncogenes, the genes that produce cancer, are only two aspects. Proto-oncogenes are found in normal cellular DNA and are believed to play regulatory roles in differentiation and development. Oncoviruses, mutation of DNA and chromosomal damage can activate proto-oncogenes and cause malignant change. Oncogenes can render transformed cells independent of growth factors. A cell can bypass the need for outside growth factors by producing the growth factor and its receptor, thereby using an autostimulatory impetus for growth. This is autocrine growth. An oncogene can also bypass the need for growth factors by activating or modifying growth

factor receptors, or by stimulating intracellular events, such as tyrosine phosphorylation, both of which ultimately lead to cell division.

The various mechanisms by which oncogenes act provide specific targets for treatment. Specific antigrowth factor or antireceptor antibodies or antagonists could interfere with autocrine regulation. Further research on the activation of oncogenes could provide valuable insight on regulation of the growth of tumors. Ultimately, the understanding of the molecular pathogenesis of cellular transformation will be a key to the prevention and treatment of cancer.

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