Autocrine Growth Factors and Solid Tumor Malignancy

Moderator
JOHN H. WALSH, MD

Discussants
WILLIAM E. KARNES, MD; FRANK CUTTITTA, PhD; *and* AMEAE WALKER, PhD

This is an edited summary of an Interdepartmental Conference arranged by the Department of Medicine of the University of California, Los Angeles, School of Medicine. The Director of Conferences is William M. Pardridge, MD, Professor of Medicine.

The ability of malignant cells to escape the constraints that normally regulate cell growth and differentiation has been a primary focus of attention for investigators of cancer cell biology. An outcome of this attention has been the discovery that the protein products of oncogenes play a role in the activation of growth signal pathways. A second outcome, possibly related to abnormal oncogene expression, has been the discovery that malignant cells frequently show an ability to regulate their own growth by the release of autocrine growth modulatory substances. Most important, the growth of certain malignant cell types has been shown to depend on autocrine growth circuits. A malignant tumor whose continued growth depends on the release of an autocrine growth factor may be vulnerable to treatment with specific receptor antagonists or immunoneutralizing antibodies designed to break the autocrine circuit. Information is rapidly emerging concerning autocrine growth factors in selected human solid tissue malignancy.

(Walsh JH, Karnes WE, Cuttitta F, Walker A: Autocrine growth factors and solid tumor malignancy. West J Med 1991 Aug; 155:152-163)

JOHN H. WALSH, MD*: Communication between cells is accomplished by the release of "messenger" substances that are delivered to remote target cells through the circulation (endocrine) or to neighboring cells through diffusion (paracrine). When the secreting cell is also the target cell, the term "autocrine" is used (Figure 1). An autocrine substance that stimulates proliferation might cause clonal expansion and tumor formation if negative proliferative influences are not operative or if the cell's life span becomes prolonged. In fact, autocrine-stimulated proliferation has been shown to be a common characteristic of malignant cells in culture. This finding has stimulated tremendous interest in the role of autocrine growth factors in the development and maintenance of malignancy.[1]

Background

WILLIAM E. KARNES, MD†: Malignant cells in culture show several features consistent with autocrine- or paracrine-mediated proliferation: they proliferate in serum-free media containing few (if any) exogenous growth factors,[2] they often secrete growth-promoting peptides, and their growth is enhanced by high plating density.[3] The proof of autocrine-mediated proliferation requires a demonstration that the clones of cells coexpress the putative autocrine growth factor and its specific receptor. In addition, the cells should proliferate in the absence of the exogenous addition of the putative autocrine growth factor and show inhibition of proliferation in the presence of a specific growth factor-receptor antagonist or an immunoneutralizing antibody against the growth factor. Table 1,[4-29] prepared by Frank Cuttitta, MD (author of the Small-cell Lung Cancer section), lists several human malignant tumors, each accompanied by a list of growth factors that have been suggested to be autocrine growth factors.

Can Growth Factors Initiate Malignancy?

Considerable effort has been directed toward determining if autocrine-mediated proliferation is sufficient to promote

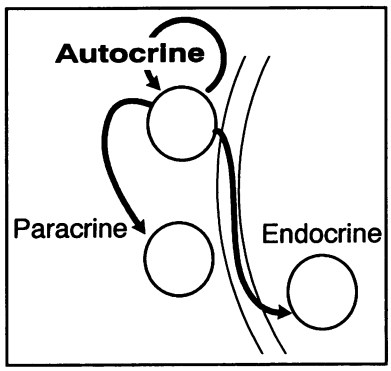

Figure 1.—Communication between cells by soluble factors may occur through the circulation to remote target cells (endocrine) or by diffusion to nearby cells (paracrine). When the secreting cell is the target cell, the term "autocrine" is used.

*Department of Medicine, Division of Gastroenterology/CURE, Wadsworth VA, Los Angeles, California.

†Department of Medicine, Division of Gastroenterology/CURE, Wadsworth VA, Los Angeles, California.

Reprint requests to John H. Walsh, MD, CURE/Wadsworth Department of Veterans Affairs, Bldg 115, Rm 115, Wilshire and Sawtelle Blvds, Los Angeles, CA 90073.

This work was supported by National Institutes of Health grant DK17328 and Department of Veterans Affairs/Department of Defense (DOD) Research Funds (Drs Walsh and Karnes); a Department of Veterans Affairs Associate Investigator Award (Dr Karnes); a grant from the G. Harlod and Leila Y. Mathers Charitable Foundation (Dr Cuttitta); and in part by a National Institutes of Health grant AM 28534 and, more recently, ACS BC 562 (Dr Walker).

ABBREVIATIONS USED IN TEXT

EGF = epidermal growth factor
GRP = gastrin-releasing peptide
IGF = insulinlike growth factor
PDGF = platelet-derived growth factor
TGF = transforming growth factor

and maintain malignancy. In the presence of transforming growth factors (TGF-α and TGF-β), normal rat kidney cells become anchorage independent (transformed), a characteristic of malignant cells.[30] When these cells are imparted with an autocrine growth circuit by transfection with an "activated" gene encoding TGF-α (constitutively activated by a promoter sequence), they become transformed in the presence of exogenous TGF-β alone.[31] Similarly, when NIH 3T3 fibroblasts are imparted with a platelet-derived growth factor (PDGF) autocrine circuit by transfection with an activated PDGF gene, a transformed phenotype is produced.[32] Using similar techniques, epidermal growth factor (EGF)-receptor overexpression has also been shown to induce transformation in NIH 3T3 cells in the presence of EGF.[33,34] Finally, and perhaps most significant, the activated expression of TGF-α in transgenic mice has been shown by independent laboratories to induce malignant changes in breast and hepatic epithelium.[35-37] Together these data suggest that autocrine growth circuits may be sufficient to induce and maintain malignancy.

Autocrine-mediated proliferation, however, is not limited to malignant cells. The proliferation of nontumorigenic cell lines derived from normal breast epithelium[38] and a cell line derived from a colonic adenoma[39] has been shown to depend on intact TGF-α- or EGF-receptor autocrine loops. In addition, TGF-α and its receptor are ubiquitous products of many normal tissues, including skin keratinocytes,[40] macrophages,[41] and epithelium of the entire gastrointestinal tract.[42,43] Autocrine-mediated proliferation, therefore, may be a normal phenomenon important in growth, development, and wound repair. Malignancy, however, may be an outcome of the autocrine secretion of growth factors by cells that lack (or lose) the ability to govern the growth signal pathway.

Growth Factors, Oncogenes, Tumor Suppressor Genes, and the Growth Signal Pathway

Progress in the understanding of the role of autocrine growth factors in malignancy has paralleled an explosion of knowledge concerning the genes associated with malignancy: oncogenes and tumor suppressor genes. This progress has led to new insights into the process of malignant transformation. Malignant transformation occurs in response to various defects affecting the growth signal pathway, whether occurring at the level of growth factor-receptor interaction, second messenger systems, or at the level of the nucleus (Figure 2).

Oncogenes are dominantly acting genes that can induce transformation of normal cells into malignant phenotypes (see reviews by Klein, Weinberg, and Bishop[44-46]). They are mutant forms of normal genes that encode protein constituents of the growth signal pathway (Figure 2). For example, growth factors are represented by v-*sis*, which encodes a PDGF-like peptide; v-*erb-b*, which encodes a truncated EGF receptor-like molecule with tyrosine kinase activity (a particularly common characteristic of oncogenes and normal growth factor receptors); the *ras* family of genes encoding guanosine triphosphate-binding proteins, which may be involved in second messenger systems; and, finally, a large group of nuclear oncogenes *(myc, fos, jun)* that encode proteins which seem to regulate the expression of genes important in differentiating and initiating DNA synthesis and cell division. For the most part, oncogene products are constitutively activated forms of their normal counterparts and likely act by causing a short circuit of the growth signal pathway.

Tumor suppressor genes are also associated with malignancy. In contrast to oncogenes, tumor suppressor genes are recessively acting genes; the loss or destructive mutation of both alleles is associated with the development of malignancy (see reviews by Klein and Hansen and Cavenee.[44,47]). Examples of tumor suppressor genes include the RB gene, the loss of which is associated with retinoblastoma,[47] and the p53 gene, the loss of which is associated with colorectal carcinomas.[48] The protein products of tumor suppressor genes seem to play an important role in normal cells to prevent malig-

TABLE 1.—*Possible Autocrine or Paracrine Growth Factors of Human Malignant Tumors*

Peptide	*Tumor*	*Study*
Gastrin-releasing peptide	Small-cell lung cancer	Cuttitta et al, 1985[4]
Insulinlike growth factor I	Fibrosarcoma	DeLarco and Todaro, 1978[5]
	Osteosarcoma	Blatt et al, 1984[6]
	Colon carcinoma	Tricoli et al, 1986[7]; Culouscou et al, 1987[8]
	Liposarcoma	Tricoli et al, 1986[7]
	Breast carcinoma	Furlanetto and DiCarlo, 1984[9]; Huff et al, 1986[10]
	Non-small-cell lung cancer	Minuto et al, 1988[11]; Minuto et al, 1986[12]
	Small-cell lung cancer	Nakanishi et al, 1988[13]
Insulinlike growth factor II	Neuroblastoma	El-Badry et al, 1988[14]
	Leiomyosarcoma	Daughaday et al, 1988[15]
Interleukin-1	Monocytic leukemia	Furukawa et al, 1987[16]
	Acute myelocytic leukemia	Cozzolino et al, 1989[17]
Interleukin-2	T-cell lymphoma	Duprez et al, 1985[18]
Interleukin-6 and B-cell stimulating factor-2	Multiple myeloma	Kawano et al, 1988[19]
Platelet-derived growth factor	Glioblastoma	Hermansson et al, 1988[20]
Transferrin and transferrinlike proteins	Small-cell lung cancer	Nakanishi et al, 1988[21]; Vostres et al, 1988[22]
Transforming growth factor-α	Breast carcinoma	Dickson et al, 1986[23]; Dickson et al, 1986[24]
	Colon carcinoma	Coffey et al, 1986[25]
	Pancreatic carcinoma	Smith et al, 1987[26]
	Non-small-cell lung cancer	Siegfried, 1987[27]
Transforming growth factor-β	Colon cancer	Coffey et al, 1987[28]
	Breast cancer	Knabbe et al, 1987[29]

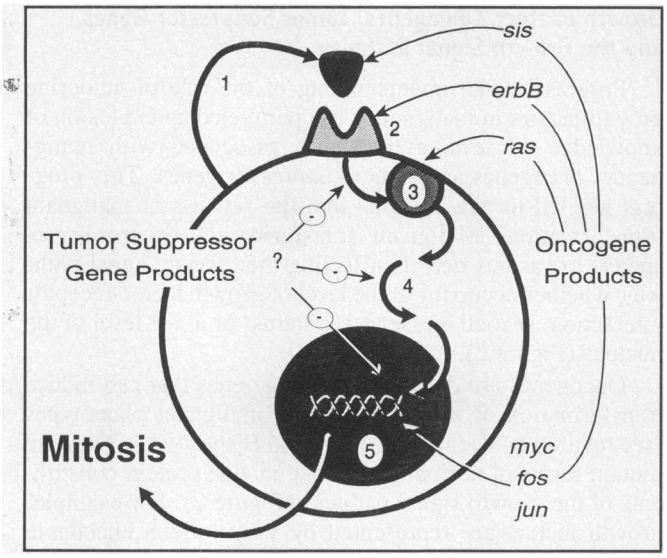

Figure 2.—The relation is shown between representative oncogene products and various steps of the growth signal pathway. Growth factors ① interact with cell surface receptors, ② leading to the activation of second messengers ③ that relay information through the cytoplasm ④ into the nucleus ⑤. Oncogene products are represented at all levels of the growth signal pathway (see text for more detail). Tumor suppressor genes may play an important regulatory role.

nancy, perhaps by tightly regulating key elements of the growth signal pathway.

Autocrine Growth Loops May Be Targets for Therapeutic Intervention

Regardless of the relative roles of oncogenes, tumor suppressor genes, and autocrine growth loops in the process of malignant transformation, autocrine-stimulated proliferation is a common characteristic of malignant cells and is potentially vulnerable to pharmacologic intervention. A malignant tumor whose continued proliferation depends on an intact autocrine growth loop may be vulnerable to treatment strategies that use specific receptor antagonists or growth factor antibodies. This approach has led to phase I clinical trials using monoclonal antibodies to gastrin-releasing peptide (GRP) in patients with advanced small-cell lung cancer.[49]

Autocrine Growth Factors Regulating Proliferation of Pulmonary Malignancy

FRANK CUTTITTA, PHD*: The primary research of our laboratory has focused on identifying and characterizing the autocrine growth factors of human malignant tumors, with an emphasis on small-cell and non–small-cell lung cancers. By understanding tumor cell biology and the factors that regulate growth, we can devise rational therapeutic strategies against malignant disease. We have used three approaches to study the autocrine growth factors of lung cancers:

- *Exogenous requirements for growth factors*

Biologically defined factors are introduced to established cancer cell lines, and their growth-regulating effects (stimulation or suppression) are examined. The responding tumor cells then are analyzed for their ability to produce these biologically active mediators. An example is our studies with insulinlike growth factors (IGFs) and with transferrin, as described subsequently.

- *Endogenous production of growth factors*

When a tumor cell line has shown an abnormal production of a specific peptide hormone, its growth regulatory properties on that same tumor cell line are examined. The growth-promoting properties of gastrin-releasing peptide on human small-cell lung cancer were identified in this manner.

- *Molecular genetic approach to identify new factors*

Genes that encode the transcription of defined growth factors also contain sequence codons for additional peptide products with possible biologic functions. We have identified at least three new peptide products that have growth-promoting effects for normal and malignant cells.[50] These novel factors are encoded within pro-GRP and pro-IGF-I transcripts downstream from the genes' major known peptide products.[51,52]

Gastrin-Releasing Peptide (Mammalian Bombesin) as an Autocrine Growth Factor in Human Small-cell Lung Cancers

Small-cell lung cancer is a highly metastatic neuroendocrine tumor that produces various peptide hormones which can lead to various paraneoplastic syndromes associated with this disease.[53] Under cell culture conditions, small-cell lung cancer cell lines produce arginine vasopressin, adrenocorticotropin, calcitonin, parathyroid hormone-like peptides, luteinizing hormone, human chorionic gonadotropin, IGF-I, oxytocin, neurophysin, estradiol, somatostatin, glucagon, prolactin, endorphin, physalaemin, substance P, and GRP.[54,55] Gastrin-releasing peptide, the mammalian counterpart to the amphibian peptide bombesin, is the first peptide hormone that has been definitively proved to be an autocrine growth regulator of human cancer.[4,56]

Gastrin-releasing peptide has been shown to be an autocrine regulator of human small-cell lung cancer by a series of experimental studies (Table 2).[4,54,57-66] A major factor in the success of these studies was the development of a monoclonal antibody, 2A11, that reacted with the biologically active carboxyl-terminal heptapeptide region of bombesin and GRP.[4] The antibody was exquisitely selective in its binding recognition site and reacted only with biologically active amidated peptide. Antibody 2A11 blocked growth stimulation produced by exogenous GRP in tissue culture and neutralized the biologically active GRP produced by cultured small-cell lung cancer cells. The antibody also caused transplanted small-cell lung cancer tumors in nude mice to shrink, whereas another mouse immunoglobulin G1 antibody raised against an indifferent antigen, MOPC-21, had no effect.

Gastrin-releasing peptide gene expression in fetal and adult lung is confined to a distinct type of pulmonary endocrine cell known as the Kulchitsky cell. This cell is located in the base of normal bronchial epithelium interdigitated between columnar cells.[67,68] Clusters of such cells, known as neuroepithelial bodies, are arranged as apical outcroppings in the bronchial lumen and have been proposed as the progenitor cells for small-cell lung cancer.[69,70]

The increased production of GRP in small-cell lung cancer tumors and cell lines was confirmed in several laboratories.[54,57-59] Subsequently the complementary DNA encoding prepro-GRP was isolated from small-cell lung cancer lines.[71] The gene has been mapped to human chromosome 18q21.[72] The messenger RNA for GRP is subjected to

*Department of Medicine, Uniformed Services, University of the Health Sciences, and NCI-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, Maryland.

TABLE 2.—*Studies of the Role of Gastrin-Releasing Peptide (GRP) in Human Small-cell Lung Cancer*

Results	*Study*
GRP was shown to be an endogenous product of small-cell lung cancer	Moody et al, 1981[54]; Wood et al, 1981[57]; Yamaguchi et al, 1983[58]; Erisman et al, 1982[59]
GRP was found to be actively secreted by small-cell lung cancer	Moody et al, 1983[60]; Korman et al, 1986[61]; Sorenson et al, 1982[62]
Small-cell lung cancer was found to express high affinity receptors of GRP	Moody et al, 1983[63]; Moody et al, 1985[64]
Exogenously administered GRP stimulated clonigenic growth of cultured small-cell lung cancer	Carney et al, 1987[65]
Endogenously produced GRP was shown to induce autoproliferation of small-cell lung cancer	Cuttitta et al, 1985[4]
A monoclonal antibody that immunoneutralizes the growth-promoting effects of exogenous GRP and bombesin on small-cell lung cancer neutralized the growth factors released into culture medium by the same cell lines	Cuttitta et al, 1985[4]
The same monoclonal antibody blocked growth of tumors of small-cell lung cancer transplanted into nude mice	Cuttitta et al, 1985[4]
Competitive antagonist peptides that interact with the GRP receptor but produce no biologic response prevented interaction of GRP with its receptor and inhibited small-lung cancer growth in vitro	Trepel et al, 1988[66]

alternative splicing, resulting in the formation of three different mRNA products.[71] All three mRNAs code for precursor molecules with a common GRP region but differ as to their downstream amino acid composition on the carboxyl-terminal side. One alternatively spliced precursor product (pro-GRP II) differs from the largest gene product (pro-GRP I) by a 7-amino acid truncation due to a 21-base deletion in its mRNA. The shortest GRP message has a 19-base deletion, which in turn leads to a frame shift in amino acid coding that results in the formation of an entirely new peptide at the carboxyl terminus (pro-GRP III). Polyclonal antibodies have been raised against both of these carboxyl-terminal amino acid sequences and used to characterize prepro-GRP expression in normal and malignant lung tissues.[73] Immunohistochemical studies have shown that both peptide I or II and peptide III gene products are produced by normal and malignant pulmonary endocrine cells.[73]

Peptides that result from the alternate splicing of GRP mRNA have been isolated and characterized from small-cell lung cancer cell lines.[52] One of these was the C-terminal tetradecapeptide of prepro-GRP III. A synthetic analogue of this peptide was shown to bind with high affinity (2 nmol per liter) to specific receptors on small-cell lung cancer cells that did not interact with GRP. There is some preliminary evidence that this peptide may be another growth-stimulating product of the GRP gene for small-cell lung cancer.

A 78-kd protein has been putatively identified as the GRP receptor in small-cell lung cancer.[64] Small-cell lung cancer cells have a relatively low number of receptors on their surface, on the order of 1,000 to 3,000 per cell, with a ligand binding constant of approximately 1 nmol per liter. Neither the tumor receptor nor the normal receptor has yet been cloned and sequenced.

The growth-stimulating properties of GRP on small-cell lung cancer usually show a narrow concentration range for the optimal stimulation of clonigenic growth with an inhibition of growth by supramaximal doses.[65] Optimal concentrations of GRP for stimulating the growth of small-cell lung cancer cells usually are 10 to 100 nmol per liter. Gastrin-releasing peptide has also been shown to stimulate the growth of normal bronchial epithelial cells in culture.[74]

Insulinlike Growth Factor I in Small-cell Lung Cancer

Insulin is known to be a factor that enhances the growth of small-cell lung cancer and many other human tumor cell lines.[75] In humans, insulinlike growth factors (IGF-I and IGF-II) are products of separate genes and interact with high-affinity receptors distinct from the high-affinity insulin receptor.[76-83] Insulinlike growth factors I and II are known to play an important role in regulating mammalian growth during fetal development.[84] Several studies with primary human non–small-cell lung cancers showed increased levels of IGF-I.[11,12]

We studied the effects of insulin on small-cell lung cancer and found that the proliferation of growth it induced was not mediated through the high-affinity insulin receptor.[13] Using reagents that had been applied to study the regulation of growth of 3T3 cells,[85,86] we found that a monoclonal antibody to the IGF-I receptor, α-IR-3, neutralized the insulin-induced growth of small-cell lung cancer without binding to the insulin receptor. Recombinant IGF-I was shown to be more potent than insulin in stimulating the growth of small-cell lung cancer in vitro, and this stimulation was blocked by monoclonal antibody α-IR-3,[13] providing additional evidence that the IGF-I receptor was dominant in stimulating the growth of small-cell lung cancer by both insulin and IGF-I.

To establish whether or not IGF-I had an autocrine stimulatory role in small-cell lung cancer, we developed antibodies against IGF-I and IGF-I gene-related peptides predicted from cDNA analysis of IGF-I to be located downstream (carboxyl-terminal) to IGF-I in prepro-IGF-I. These polyclonal antibodies were used to screen whole cell lysates of several small-cell lung cancer lines for the expression of IGF-I and IGF-I gene-related peptides. Several cell lines were found to produce these peptides. Endogenously secreted IGF-I was found to retain full biologic activity to initiate the growth of other small-cell lung cancer cell lines, and monoclonal antibody α-IR-3 lowered the growth rate of small-cell lung cancer cell lines.[13] These data imply that IGF-I produced by some small-cell lung cancer cell lines functions as an autocrine regulator of tumor cell growth.

Transferrin in Small-cell Lung Cancer

We have identified transferrin or transferrinlike protein as an additional autocrine growth factor in small-cell lung cancer.[21] This finding has been confirmed by another laboratory.[22] Our studies on transferrin were similar to studies we did with IGF-I. The initial experiments showed that small-cell lung cancer cell lines used transferrin to maintain growth. In serum-free culture conditions, transferrin is used

as the iron-binding protein to satisfy cellular cofactor demands of the electron transport system. Several small-cell lung cancer cell lines can grow in the absence of exogenous transferrin. Because there are no known biologic substitutes for transferrin, such a finding implicated the endogenous production of transferrin or a transferrinlike protein. On further investigation, it was found that the growth of small-cell lung cancer cell lines could be blocked by the use of a monoclonal antibody to the transferrin receptor, MoAb 42/6.[21,87,88] Subsequent studies have shown that small-cell lung cancer cell lines produce an 80-kd protein that shares immunologic homology with native transferrin.[22] All these data show that transferrin or other transferrinlike proteins, or both, initiate the autocrine growth of small-cell lung cancer in vitro.

Conclusions

We have shown that GRP, IGF-I, and transferrin can function as autocrine regulators of the growth of small-cell lung cancer cell lines in vitro. The autocrine growth-promoting role of GRP has also been shown in vivo. These studies have expanded our understanding of the growth regulation of small-cell lung cancer and have brought us closer to the development of new therapeutic strategies based on the interruption of autocrine growth stimulants. Current strategy has involved the use of neutralizing antibodies raised against tumor growth factors or their respective receptors to block autocrine loop mechanisms of proliferation. Future directions will include the development of specific growth factor receptor chemical antagonists and cytotoxic reagents that are targeted to growth factor receptors.

Breast Carcinoma

AMEAE M. WALKER, PhD*: The pivotal role of estrogens as promoters of human breast cancer is well established. The estrogen dependency of many breast cancers has permitted effective treatments using antiestrogens or oophorectomy. Unfortunately, by the time breast cancer reaches the stage of overt metastatic disease, estrogen dependence is frequently lost.[89] The search for an understanding of the estrogen promotion process and the mechanisms whereby most of the tumors subsequently escape estrogen dependence has led to evidence that estrogen may stimulate the proliferation of breast cancer cells by modulating the release of, and response to, autocrine and paracrine growth factors.[90]

Several autocrine growth factors have been shown to be elaborated by breast cancer cells in culture and in some cases by normal breast tissues in vitro and in vivo (Table 3). The secretion of many of these factors has been shown to be regulated by estrogen. The growth-promoting peptides, TGF-α, IGF-I, and 52K-cathepsin D, are positively regulated by estrogen, whereas the growth-inhibiting peptide, TGF-β, is negatively regulated by estrogen. Other potential autocrine growth-regulating peptides now being studied in breast cancers include IGF-II, basic fibroblast growth factor, platelet-derived growth factor, and the recently recognized growth-inhibiting peptide, mammostatin.[91]

Transforming Growth Factor-α

Of the growth-stimulating factors, TGF-α, which has received the most attention, is a 5.6-kd, 50-amino acid polypeptide that is structurally similar to EGF and probably acts through the EGF receptor. In 1984 Salomon and colleagues showed that conditioned medium from mammary tumor cells competed with EGF labeled with iodine 125 for EGF receptor binding on 3T3 fibroblasts and stimulated the growth of normal rat kidney cells in soft agar.[92] These workers also found EGF receptors on the same mammary tumor cells that released EGF-receptor binding activity. This work has been verified and extended by Dickson and associates,[23,93] who found that EGF-receptor binding activity was TGF-α. The ability of these cells to produce TGF-α and growth response to exogenous TGF-α suggests an autocrine role in mammary carcinoma. The fact that approximately 70% of primary mammary tumors express TGF-α mRNA[94] and as many as 67% possess EGF receptor[95] emphasizes the possible importance of this autocrine circuit in breast cancer.

The expression and secretion of TGF-α seem to be tightly regulated by estrogen in estrogen-sensitive cells. Several mammary tumor cell lines have been shown to produce anywhere from two to eight times more TGF-α in response to estrogen administration, whereas estrogen depletion or treatment with antiestrogens leads to much lower production,[23,94] an effect that is paralleled by the stimulation and inhibition of growth, respectively.[96] Epidermal growth factor-receptor expression also seems to be positively regulated by estrogen[97,98] and EGF.[99] The growth-inhibiting effect of antiestrogens on breast cancer cell lines can be reversed by the exogenous administration of EGF.[100] Taken together, these observations suggest that TGF-α autocrine secretion is mediated by estrogen in estrogen-dependent cell lines.

Bates and co-workers tested the dependency of estrogen-stimulated growth on the autocrine secretion of TGF-α in estrogen-sensitive cells (MCF-7).[94] Immunoneutralizing anti–TGF-α antibody and EGF-receptor antagonist antibodies each significantly inhibited estrogen-induced growth after several days. These results contrast with those of Arteaga and colleagues, who were unable to show an inhibition of estrogen-induced DNA synthesis by anti–TGF-α antibody or EGF-receptor antagonist after 18 hours using MCF-7 cells.[101] In the latter study, cells were pretreated with tamoxifen to accentuate the estrogen response. A role of TGF-α as the mediator of estrogen-induced growth of breast carcinoma was further disputed by the work of Clarke and associates.[102] They transfected MCF-7 cells with an activated TGF-α gene to induce constitutive overexpression and secretion of TGF-α. Although the transfected cell lines secreted large amounts of TGF-α, they remained estrogen-dependent and were unable to form tumors in oophorectomized nude mice. In summary, much evidence suggests an important role for TGF-α in stimulating the growth of estrogen-responsive breast carci-

TABLE 3.—*Estrogen-Responsive Growth Factors in Human Breast Carcinoma*

Peptide	Route	Growth	Estrogen Effect on Release
Transforming growth factors-α	Autocrine	↑	↑
Insulinlike growth factors I	Autocrine	↑	↑
52K-Cathepsin D	Autocrine	↑	↑
Insulinlike growth factor II	(Autocrine?)	↑	↑
Basic fibroblast growth factor	(Autocrine?)	↑	?
Platelet-derived growth factor	(Paracrine?)	↑(Stroma)	?
Transforming growth factors-β	Autocrine	↓	↓
Mammostatin	(Autocrine?)	↓	?

*Department of Biomedical Sciences, University of California, Riverside.

noma cells, although the role of TGF-α as a mediator of estrogen-induced growth is disputed.

Insulinlike Growth Factor I

Insulinlike growth factor I is a 7.6-kd polypeptide with considerable structural similarity to proinsulin. Although IGF-I is able to bind insulin receptor and stimulate many of insulin's metabolic effects, it exerts its mitogenic effect through interaction with its own specific receptor. Insulinlike growth factor I is a potent mitogen for breast cancer cells in vitro.[103] Like TGF-α, IGF-I can override the growth-inhibitory effects of tamoxifen.[96] Insulinlike growth factor-I receptors are present on most of breast carcinoma cell lines[104,105] and primary breast carcinomas,[106] whereas few, if any, IGF-I receptors are detectable in normal breast tissues.[107,108] The receptor content of IGF-I is positively correlated with estrogen receptor content in primary tumors.[106,108] Like TGF-α, IGF-I release seems to be stimulated by estrogen,[109] although estrogen may not be the primary regulator.[103]

52K-Cathepsin D

52K-Cathepsin D, a glycoprotein precursor of the 34-kd protease, cathepsin D,[110,111] is secreted by and is mitogenic for breast carcinoma cell lines. Its secretion is induced by estrogen in estrogen-dependent cell lines,[112] and it is mitogenic for the same cells in the absence of estrogen.[113] Its expression is also induced by EGF, insulin, IGF-I, and basic fibroblast growth factor.[114] In estrogen-independent cell lines, 52K-cathepsin D is constitutively expressed.[115] The mechanism by which it stimulates proliferation is unclear. It may function enzymatically to activate or inactivate co-secreted growth-promoting or -inhibiting propeptides, respectively.[116,117] Alternatively, it may exert its mitogenic effects directly. Morgan and colleagues found a high degree of structural similarity between the mannose-6-phosphate receptor and the IGF-II receptor.[118] Because cathepsin D bears mannose-6-phosphate moieties and binds mannose-6-phosphate receptors,[119] it may have the capacity to interact with IGF-II receptor to induce mitogenesis directly. Finally, cathepsin D may function to enhance tumor invasiveness by its proteolytic activities on basement membrane components.[120]

Insulinlike Growth Factor II

Insulinlike growth factor II is structurally related to IGF-I and proinsulin and binds a unique receptor.[121] Yee and associates showed that IGF-II is produced by and is mitogenic for breast cancer cells in vitro.[122] They also found that IGF-II production, like most of the other growth factors mentioned above, is induced by estrogen in estrogen-sensitive cell lines. Specific receptors and several binding proteins for IGF-II have been found in most breast carcinoma cell lines.[105] Insulinlike growth factor II mRNA is frequently expressed and produced by human mammary carcinomas and by surrounding normal tissues,[122] suggesting that this peptide may prove to be an important autocrine or paracrine growth regulator of breast carcinoma.

Fibroblast Growth Factors and Platelet-derived Growth Factor

Fibroblast growth factor-related genes have been shown to be amplified and expressed in approximately 17% of primary breast carcinomas.[123,124] Basic fibroblast growth factor stimulates the proliferation of breast carcinoma cells in vitro.[103,125] Platelet-derived growth factor-related proteins are also produced by breast cancer cells and surrounding stromal tissue but do not stimulate autogrowth of breast cancer cells.[126-128] They may contribute to tumor growth by stimulating stromal proliferation.

Transforming Growth Factor-β—A Growth Inhibitor

The fourth growth factor listed in Table 3, TGF-β, has received much attention because of its ability to inhibit the growth of many epithelial carcinomas. Transforming growth factor-β represents a family of structurally related peptides that bind to specific receptors.[129,130] In the presence of EGF, TGF-β will induce colony formation of normal rodent fibroblast cells in soft agar.[30] This growth factor is particularly interesting because, contrary to its growth-stimulating effects on normal rat kidney cells in soft agar, it has a growth-inhibiting effect on normal rat kidney cells when grown in monolayers. It is capable of stimulating or inhibiting a wide variety of cell types.[131] Growth inhibition by TGF-β is most often seen in cells of epithelial origin, including mammary tumor cells.[29]

Mammary tumor cells frequently produce TGF-β, bear receptors for TGF-β, and increase their production of TGF-β in response to antiestrogens or estrogen withdrawal.[29] In estrogen-responsive cell lines, estrogen causes a reduction in TGF-β production,[23] whereas antiestrogens or estrogen withdrawal induces TGF-β production.[29] The inhibitory effects of estrogen on the production of TGF-β and its stimulatory effects on the production of growth-promoting peptides can easily be envisioned as a mechanism explaining the estrogen dependence of mammary tumors. Other estrogen-dependent mammary carcinoma cell lines do not follow this pattern, however, as judged by the effects of estrogen on mRNA levels of TGF-α and TGF-β.[132] There are probably other mechanisms of estrogen promotion.

Escape From Estrogen Dependence

We can well envision an escape from estrogen dependence resulting from the constitutive production of autocrine growth factors or the underproduction of autocrine growth-inhibitory factors (TGF-β). In line with this hypothesis is the finding that EGF causes a decrease of estrogen-receptor levels in estrogen-sensitive cell lines.[133] Dickson and coworkers, however, found a complete lack of correlation between estrogen dependence or tumorigenicity and the overproduction of TGF-α, or the underproduction of TGF-β, in breast carcinoma cell lines.[23] In addition, estrogen receptor-positive cell lines frequently lack TGF-β receptor, and their growth is not greatly inhibited by exogenous TGF-β, whereas estrogen-negative cell lines often bear TGF-β receptor, and their growth is potently inhibited by TGF-β.[134] Finally, the TGF-α content of estrogen receptor-negative primary mammary carcinomas has been found to be reduced by 50% compared with estrogen receptor-positive tumors.[135]

Battaglia and associates reported a substantially higher incidence of EGF-receptor positivity in metastatic lesions compared with primary tumors.[136] Using immunocytochemical techniques, the same group and others found a significant inverse relation between staining for EGF receptor and estrogen receptor in primary breast tumors, respectively.[108,136,137] In other studies by Nicholson and colleagues

using immunocytochemical and radioreceptor assay techniques on tumors removed after antiestrogen therapy, a strong positive relation between a poor response to antiestrogen therapy and EGF-receptor positivity or estrogen-receptor negativity was found.[138,139]

HER-2/neu *Oncogene Products Are Related to Estrogen Independence and Poor Prognosis*

Recently the amplification of an EGF-receptor–related proto-oncogene variously known as HER-2, *neu*, or c-*erb* B2, has been seen in 25% to 30% of primary breast carcinomas.[140] The HER-2/*neu* proto-oncogene has been shown to be inversely associated with estrogen- and progesterone-receptor status[141] and negatively correlated with prognosis.[140] Presumably it encodes a growth factor receptor, but its ligand has not yet been identified.

Escape from estrogen dependence seems to be better correlated with the expression of the EGF receptor and its closely related family member (HER-2/*neu*) than to the expression of TGF-α or TGF-β. This finding does not exclude the possibility that other as-yet-unidentified growth-modulating ligands may be important in the process of estrogen escape. It does, however, emphasize that a short circuit of the growth signal pathway does not have to be autocrine. Constitutively activated receptors, second messenger systems, or primary growth signals at the level of the nucleus could lead to autonomous growth independent of extracellular modulatory substances. The complex interactions between intracellular and extracellular events in the growth signal pathway remain poorly understood.

Gastrointestinal Cancers

DR KARNES: Autocrine growth circuits in gastrointestinal cancers have been less well characterized than those of breast cancer and small-cell lung cancer. Data are emerging in support of the importance of autocrine growth factors in gastrointestinal malignant tumors. Growth factors attracting the most attention have included TGF-α, TGF-β, and gastrin. The strongest data to date involve the autocrine role of TGF-α in cancers of the esophagus, stomach, pancreas, and colon.

Transforming Growth Factor-α

Esophagus. Squamous cell carcinoma of the esophagus is associated with the amplification and overexpression of the EGF–receptor gene.[142] Tissue and cell lines derived from squamous cell carcinoma of the esophagus also have an elevated receptor number compared with other carcinomas.[143,144] Yoshida and co-workers recently showed coexpression of the EGF receptor and its ligand, TGF-α, in all six esophageal squamous carcinoma cell lines they tested.[145] Messenger RNA and protein of EGF were also produced in three of the six cell lines. Monoclonal antibodies against EGF and TGF-α inhibited DNA synthesis in one of these cell lines, suggesting that EGF and TGF-α functioned as autocrine growth factors.

Stomach. As in squamous cell carcinoma of the esophagus, gastric carcinoma tumor specimens and cell lines almost universally express EGF receptors.[146-148] Similarly, the EGF-receptor ligands, TGF-α and EGF, are also produced by gastric carcinoma cell lines.[149,150] The level of EGF-like immunoreactivity in gastric carcinoma tissues has been associated with a poor prognosis.[149] An autocrine role of TGF-α and EGF in gastric carcinoma has been suggested by Yoshida and colleagues in work similar to that described in esophageal carcinoma cell lines.[151] They found that TGF-α and EGF receptor were coexpressed in all 7 gastric carcinoma cell lines and in all 15 gastric carcinoma tumors they examined. Furthermore, they showed that antibodies against TGF-α and EGF inhibited DNA synthesis of cultured cell lines.

Pancreas. Two pancreatic carcinoma cell lines have been shown by Smith and associates to possess EGF receptors, express and release TGF-α, and proliferate in response to exogenous EGF or TGF-α (TGF-α was 10 to 100 times more potent than EGF).[26] They further noted that cells that produced TGF-α also overexpressed EGF receptor, suggesting a superagonist autocrine cycle involving TGF-α and EGF receptor. These authors, however, did not extend their study to show that the cells could grow in the absence of exogenous EGF or TGF-α and that growth under these conditions could be specifically inhibited by the addition of neutralizing anti–TGF-α antibody or EGF-receptor antagonist.

Colon. Colon cancer cell lines often show a density-dependent stimulation of cell growth, and conditioned media from these lines can stimulate the growth of other cell lines, implying the production and secretion of growth factors into the conditioned media.[152] In most colon cancer cell lines that have been examined, TGF-α and EGF or TGF-α receptor are coexpressed.[28,152-155] The EGF-receptor antagonist, anti–EGF-receptor monoclonal antibody 425, has been shown by Rodeck and associates to inhibit the growth of a human colon carcinoma cell line in nude mice.[156]

In our laboratory, we have shown that the colon carcinoma cell line, SNU-C1, coexpresses TGF-α and its receptor and releases TGF-α into serum-free conditioned medium.[157] We have further shown that this cell line has the ability to proliferate autonomously in serum-free medium containing no exogenous growth factors and shows a significant proliferative response to exogenously added TGF-α, EGF, or serum-free medium conditioned by SNU-C1 cells. Using an EGF receptor-blocking monoclonal antibody, we have shown that the autonomous proliferation of SNU-C1 cells is entirely dependent on EGF-receptor activation by an autocrine EGF-receptor ligand, most likely TGF-α.[157] Future studies in additional cell lines will be needed to determine if the dependency of proliferation on the autocrine secretion of TGF-α is a common phenomenon in colon carcinomas.

Transforming Growth Factor-β

Almost all published work with TGF-β in gastrointestinal malignancy has been in colon carcinoma. Anzano and co-workers found TGF-β in 16 of 18 colon cancer cell lines.[152] In addition, colon cancer cell lines frequently possess specific high-affinity TGF-β receptors and have a growth-inhibitory response to TGF-β.[158] Interestingly, the growth-inhibitory response to TGF-β was more apparent in well-differentiated cell lines than in those more poorly differentiated. Growth inhibition by TGF-β has been verified by other investigators[159,160] but has not always been found.[25] More work is needed to establish the role of TGF-β as an autocrine growth-inhibitory factor in gastrointestinal cancers.

Gastrin

Recent evidence suggests a role of gastrin as an endocrine or paracrine (autocrine) growth stimulant of colon and gas-

TABLE 4.—*Functions of Elevated Extracellular Prolactin*

Function	*Time*	*Study*
Inhibits further prolactin secretion	Immediate	Kadowacki et al, 1984[173]
Results in the reuptake of already secreted prolactin	Immediate	Kadowacki et al, 1984[173]; Giss and Walker, 1985[174]
Promotes the degradation of newly synthesized prolactin	After 4 h	Kadowacki et al, 1984[173]
Inhibits prolactin synthesis	12 to 24 h	Walker et al, 1988, unpublished data

tric carcinomas. In addition to gastrin's prosecretory effects on the gastric mucosa, it also has been reported to have a trophic effect on normal gastric and colonic mucosa and on human colon carcinoma cell lines in nude mouse xenografts.[161-163] The proliferation of some colon cancer cell lines, particularly those that are well differentiated, is stimulated by exogenous gastrin.[164,165] Several colon and gastric carcinoma cell lines are known to have gastrin receptors,[166-169] and some colon carcinoma cell lines also express gastrin mRNA, suggesting a possible autocrine role of gastrin.[170] The gastrin antagonist, proglumide, and gastrin antibodies inhibit the growth of gastrin-responsive colon cancer cells in culture and in xenografts.[165,170,171] Although a role of circulating gastrin in colon cancer development is suggested by evidence that serum gastrin values are increased in patients with colon cancer,[172] there are few studies of the mechanism of action of gastrin to stimulate colon cancer proliferation.

Autocrine Regulation of Pituitary Tumor Growth and Secretion by Prolactin Isoforms

DR WALKER: For several years we have been interested in the autocrine regulation of prolactin secretion.* This interest has expanded to include the autocrine regulation of pituitary cell differentiation and proliferation. By autocrine regulation of prolactin secretion, we mean the ability of already secreted prolactin to inhibit further prolactin secretion from the same or an equivalent cell in the pituitary. Table 4 lists the results we obtained using primary cultures of rat anterior pituitary cells as a model system.[173,174]

One of the puzzling findings of these studies was that although autocrine regulation led to lysosomal degradation of newly synthesized prolactin,[173] the prolactin taken back up by the cells was never deposited in lysosomes.[174] Because the cell was able to distinguish between these two prolactins, we studied intracellular versus extracellular forms of prolactin. Many prolactin variants have been described.[175] For the purposes of autocrine regulation, only the charge variants of monomer prolactin seem to be important. These have the same molecular weight but vary in their degree of net negative charge. They have been described for all species examined, including humans.[176]

Inside pituitary cells there are three to four of these forms, called isoforms. Isoform 2 is always the predominant form, although the ratios of the forms differ under different physiologic circumstances.[177] The major forms in the medium are isoforms 2, 3, and 3′. In the medium, forms 3 and 3′ regularly appear as greater percentages of the total isoform amount than they do inside the cells. This finding suggested the possibility that 3 or 3′, or both were responsible for autocrine regulation and that form 2 would be the newly synthesized form directed to lysosomes. To test this hypothesis, we investigated the isoforms synthesized and secreted by a rat tumor cell line (GH3 cells[178]) that does not normally autoregulate its prolactin secretion. These cells were found to synthesize and secrete only isoform 2.[179] The tumor cells could, however, be induced to autoregulate by adding prolactin from normal sources that contained isoforms 2, 3, and 3′. The retained ability of these cells to autoregulate was first shown by Melmed and associates.[180] These results clearly suggest that isoform 2 is inactive in autocrine regulation, whereas isoforms 3 or 3′, or both, are important.[179]

In 1986 we showed phosphorylation of prolactin isoforms 3 and 3′ in intact pituitary cells.[181] Recently we have established that only isoform 2 is translated from normal pituitary messenger RNA in an in vitro system containing rough microsomes.[182,183] We might therefore conclude that isoforms 3 and 3′ are formed from isoform 2 by serial phosphorylation. The finding of prolactin kinase activity in secretory granules[182] shows that this phosphorylation does not occur until late in biosynthesis and storage, possibly even just before exocytosis of a prolactin secretory granule (Figure 3).

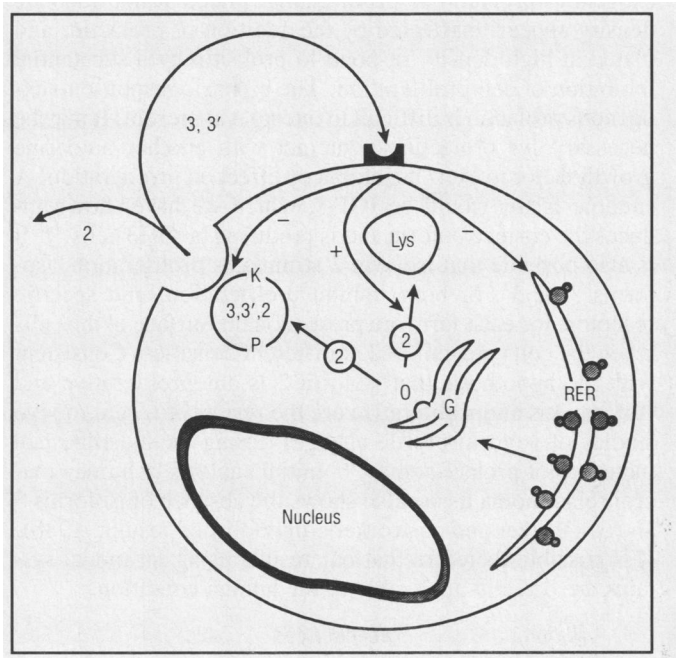

Figure 3.—A schematic representation of prolactin isoform biosynthesis, secretion, and secretory autocrine activities is given. Prolactin is synthesized on the rough endoplasmic reticulum **(RER)** as isoform 2. Isoform 2 moves through the Golgi complex **(G)** where it is packaged into small immature secretory granules. Newly synthesized granules can be diverted for digestion in a lysosome **(Lys)**, should autocrine inhibition of secretion be operative, or they can be permitted to mature until they contain isoform 3 and 3′. Secretion of all 3 isoforms occurs, but for secretory control only isoforms 3 or 3′ or both reinteract with the cell through the autoreceptor. Interaction with the autoreceptor or subsequent internalization of prolactin, or both, inhibit prolactin release, stimulate lysosomal digestion of newly synthesized prolactin, and inhibit prolactin synthesis. Kinase **(K)** phosphorylates prolactin, and phosphatase **(P)** removes phosphate.

*W. F. Young, Jr, MD, E. R. Laws, Jr, MD, and B. Scheithauer, MD, of the Mayo Clinic, Rochester, Minnesota, have collaborated with the author in the study of human prolactinoma tissue.

Having established that the autocrine regulation of prolactin secretion could be induced in the tumor cells by using the correct isoform, we wondered whether this induction resulted in any changes in tumor cell ultrastructure. Normal prolactin-secreting anterior pituitary cells are highly differentiated cells. They contain abundant and regularly arrayed rough endoplasmic reticulum, a large and polarized Golgi apparatus, and an abundance of prolactin storage granules.[184] The GH3 cells and most prolactin-secreting cell lines and tumors, on the other hand, contain sparse and irregular rough endoplasmic reticulum, dispersed Golgi elements, and few (sometimes none) small secretory granules.[185] The initiation of autocrine regulation in the tumor cells resulted in the development of more rough endoplasmic reticulum, an organized and polar Golgi apparatus, and the storage of many more prolactin granules.[186] Because granulation of these cells occurs by co-treatment with estrogen, insulin, and EGF,[187] we tested these hormones for their ability to induce isoforms 3 and 3′. Insulin (as used by Scammel and colleagues[187] at 300 nmol per liter) and IGF-I and IGF-II at 10 and 100 ng per ml, respectively, were each able to induce the production of isoforms 3 and 3′.[188] We tentatively concluded that the induction of a more normal phenotype in the tumor cells, whether initiated by prolactin or growth factors, operates by the autocrine route.

With the induction of a phenotype geared towards secretion, rather than cell division, do prolactin autocrine effects have any influence on cell proliferation? GH3 cells plated at low cell density (that used in many laboratories) respond to exogenous prolactin by proliferating, plated at intermediate density appear unaffected by the addition of prolactin, and plated at high density respond to prolactin by a substantial inhibition of cell proliferation. The biphasic response to exogenous prolactin is difficult to interpret at present. It may be necessary for prolactin to interact with another autocrine growth factor to exert its inhibitory effect on proliferation. A suitable factor would be IGF-I, which we have shown induces the correct isoform and is produced by GH3 cells.[189] It is also possible that isoform 2 stimulates proliferation, isoforms 3 or 3′, or both, inhibit proliferation, and specific receptors for each form are present on the surface of the cells when the cells are cultured at different densities. Consistent with the hypothesis that isoform 2 is autoproliferative and isoform 3 is antiproliferative are the results of recent in vivo studies of isoform profile changes during diethylstilbestrol induction of prolactinomas.[190] Initial analysis of human macroprolactinoma tissue also shows the absence of isoforms 3 and 3′ (Walker and co-workers, unpublished results, 1988). It is possible therefore that our results using rat model systems are directly applicable to the human condition.

REFERENCES

1. Sporn MB, Roberts AB: Autocrine, paracrine and endocrine mechanisms of growth control. Cancer Surv 1985; 4:627-632

2. Mulder KM, Childress-Fields KE: Characterization of a serum-free culture system comparing growth factor requirements of transformed and untransformed cells. Exp Cell Res 1990; 188:254-261

3. Browder TM, Dunbar CE, Nienhuis AW: Private and public autocrine loops in neoplastic cells. Cancer Cells 1989; 1:9-17

4. Cuttitta F, Carney DN, Mulshine J, et al: Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. Nature 1985; 316:823-826

5. DeLarco JE, Todaro GJ: A human fibrosarcoma cell line producing multiplication stimulating activity (MSA)-related peptides. Nature 1978; 272:356-358

6. Blatt J, White C, Dienes S, Friedman H, Foley TP Jr: Production of an insulin-like growth factor by osteosarcoma. Biochem Biophys Res Commun 1984; 123:373-376

7. Tricoli JV, Rall LB, Karakousis CP, et al: Enhanced levels of insulinlike growth factor messenger RNA in human colon carcinomas and liposarcomas. Cancer Res 1986; 46:6169-6173

8. Culouscou JM, Remacle-Bonnet M, Garrouste F, Marvaldi J, Pommier G: Simultaneous production of IGF-I and EGF competing growth factors by HT-29 human colon cancer line. Int J Cancer 1987; 40:646-652

9. Furlanetto RW, DiCarlo JN: Somatomedin-C receptor and growth effects in human breast cells maintained in long-term tissue culture. Cancer Res 1984; 44:2122-2128

10. Huff KK, Kaufman D, Gabbay KH, Spencer EM, Lippman ME, Dickson RB: Secretion of an insulin-like growth factor-I-related protein by human breast cancer cells. Cancer Res 1986; 46:4613-4619

11. Minuto F, Del Monte P, Barreca A, Alama A, Cariola G, Giordano G: Evidence for autocrine mitogenic stimulation by somatomedin-C/insulin-like growth factor I on an established human lung cancer cell line. Cancer Res 1988; 48:3716-3719

12. Minuto F, Del Monte P, Barreca A, et al: Evidence for an increased somatomedin-C/insulin-like growth factor I content in primary human lung tumors. Cancer Res 1986; 46:985-988

13. Nakanishi Y, Mulshine JL, Kasprzyk PG, et al: Insulin-like growth factor-I can mediate autocrine proliferation of human small cell lung cancer cell lines in vitro. J Clin Invest 1988; 82:354-359

14. El-Badry OM, Romanus JA, Helman LJ, Cooper MJ, Rechler MM, Israel MA: Autonomous growth of a human neuroblastoma cell line is mediated by insulin-like growth factor II. J Clin Invest 1989; 84:829-839

15. Daughaday WH, Emanuele MA, Brooks MH, Barbato AL, Kapadia M, Rotwein P: Synthesis and secretion of insulin-like growth factor II by a leiomyosarcoma with associated hypoglycemia. N Engl J Med 1988; 319:1434-1440

16. Furukawa Y, Ohta M, Miura Y, Saito M: Interleukin-1 derived from human monocytic leukemia cell line JOSK-I acts as an autocrine growth factor. Biochem Biophys Res Commun 1987; 147:39-46

17. Cozzolino F, Rubartelli A, Aldinucci D, et al: Interleukin 1 as an autocrine growth factor for acute myeloid leukemia cells. Proc Natl Acad Sci USA 1989; 86:2369-2373

18. Duprez V, Lenoir G, Dautry-Varsat A: Autocrine growth stimulation of a human T-cell lymphoma line by interleukin 2. Proc Natl Acad Sci USA 1985; 82:6932-6936

19. Kawano M, Hirano T, Matsuda T, et al: Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 1988; 332:83-85

20. Hermansson M, Nistér M, Betsholtz C, Heldin CH, Westermark B, Funa K: Endothelial cell hyperplasia in human glioblastoma: Coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. Proc Natl Acad Sci USA 1988; 85:7748-7752

21. Nakanishi Y, Cuttitta F, Kasprzyk PG, et al: Growth factor effects on small cell lung cancer cells using a colorimetric assay: Can a transferrin-like factor mediate autocrine growth? Exp Cell Biol 1988; 56:74-85

22. Vostrejs M, Moran PL, Seligman PA: Transferrin synthesis by small cell lung cancer cells acts as an autocrine regulator of cellular proliferation. J Clin Invest 1988; 82:331-339

23. Dickson RB, Bates SE, McManaway ME, Lippman ME: Characterization of estrogen responsive transforming activity in human breast cancer cell lines. Cancer Res 1986; 46(Pt 1):1707-1713

24. Dickson RB, McManaway ME, Lippman ME: Estrogen-induced factors of breast cancer cells partially replace estrogen to promote tumor growth. Science 1986; 232:1540-1543

25. Coffey RJ Jr, Shipley GD, Moses HL: Production of transforming growth factors by human colon cancer lines. Cancer Res 1986; 46:1164-1169

26. Smith JJ, Derynck R, Korc M: Production of transforming growth factor alpha in human pancreatic cancer cells: Evidence for a superagonist autocrine cycle. Proc Natl Acad Sci USA 1987; 84:7567-7570

27. Siegfried JM: Detection of human lung epithelial cell growth factors produced by a lung carcinoma cell line: Use in culture of primary solid lung tumors. Cancer Res 1987; 47:2903-2910

28. Coffey RJ Jr, Goustin AS, Soderquist AM, et al: Transforming growth factor alpha and beta expression in human colon cancer lines: Implications for an autocrine model. Cancer Res 1987; 47:4590-4594

29. Knabbe C, Lippman ME, Wakefield LM, et al: Evidence that transforming growth factor-beta is a hormonally regulated negative growth factor in human breast cancer cells. Cell 1987; 48:417-428

30. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB: New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues. Proc Natl Acad Sci USA 1981; 78:5339-5343

31. Watanabe S, Lazar E, Sporn MB: Transformation of normal rat kidney (NRK) cells by an infectious retrovirus carrying a synthetic rat type alpha transforming growth factor gene. Proc Natl Acad Sci USA 1987; 84:1258-1262

32. Gazit A, Igarashi H, Chiu IM, et al: Expression of the normal human sis/PDGF-2 coding sequence induces cellular transformation. Cell 1984; 39:89-97

33. Velu TJ, Beguinot L, Vass WC, et al: Epidermal-growth-factor-dependent tranformation by a human EGF receptor proto-oncogene. Science 1987; 238:1408-1410

34. Di Fiore PP, Pierce JH, Fleming TP, et al: Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. Cell 1987; 51:1063-1070

35. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC: Overexpression of TGF-alpha in transgenic mice: Induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 1990; 61:1121-1135

36. Jhappan C, Stahle C, Harkins RN, Fausto N, Smith GH, Merlino GT: TGFα overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. Cell 1990; 61:1137-1146

37. Matsui Y, Halter SA, Holt JT, Hogan BL, Coffey RJ: Development of mammary hyperplasia and neoplasia in MMTV-TGFα transgenic mice. Cell 1990; 61:1147-1155

38. Bates SE, Valverius EM, Ennis BW, et al: Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells. Endocrinology 1990; 126:596-607

39. Markowitz SD, Molkentin K, Gerbic C, Jackson J, Stellato T, Wilson JK: Growth stimulation by coexpression of tranforming growth factor-alpha and epidermal growth factor-receptor in normal and adenomatous human colon epithelium. J Clin Invest 1990; 86:356-362

40. Coffey RJ Jr, Derynck R, Wilcox JN, et al: Production and auto-induction of transforming growth factor-alpha in human keratinocytes. Nature 1987; 328:817-820

41. Rappolee DA, Mark D, Banda MJ, Werb Z: Wound macrophages express TGF-alpha and other growth factors in vivo: Analysis by mRNA phenotyping. Science 1988; 241:708-712

42. Cartlidge SA, Elder JB: Transforming growth factor alpha and epidermal growth factor levels in normal human gastrointestinal mucosa. Br J Cancer 1989; 60:657-660

43. Malden LT, Novak U, Burgess AW: Expression of transforming growth factor alpha messenger RNA in the normal and neoplastic gastro-intestinal tract. Int J Cancer 1989; 43:380-384

44. Klein G: The approaching era of the tumor suppressor genes. Science 1987; 238:1539-1545

45. Weinberg RA: The action of oncogenes in the cytoplasm and nucleus. Science 1985; 230:770-776

46. Bishop JM: Viral oncogenes. Cell 1985; 42:23-38

47. Hansen MF, Cavenee WK: Tumor suppressors: Recessive mutations that lead to cancer. Cell 1988; 53:172-173

48. Finlay CA, Hinds PW, Levine AJ: The p53 proto-oncogene can act as a suppressor of transformation. Cell 1989; 57:1083-1093

49. Mulshine JL, Treston AM, Natale RB, et al: Autocrine growth factors as therapeutic targets in lung cancer. Chest 1989; 96(Suppl):31S-34S

50. Cuttitta F, Kasprzyk PG, Treston AM, et al: The E Domain of Insulin-like Growth Factor-IB Prohormone Contains a Novel Peptide Which Is Mitogenic for Cell Derived From Both Normal and Malignant Human Lung (Abstr). Proceedings from the Eighteenth Annual Meeting of the American Association for Cancer Research 1989; 30:67

51. Kasprzyk PG, Treston AM, Avis I, et al: Human Insulin-Growth Factor-I Gene Encodes Downstream Peptides Which Can Potentially Function as Autocrine Growth Factors for a Human Colon Cancer Cell Line (Abstr). Proceedings from the Eightieth Annual Meeting of the American Association for Cancer Research 1989; 30:61

52. Reeve JR Jr, Cuttitta F, Vigna SR, et al: Multiple gastrin-releasing peptide gene-associated peptides are produced by a human small cell lung cancer line. J Biol Chem 1989; 264:1928-1932

53. Bunn PA Jr, Minna JD: Paraneoplastic syndromes, *In* DeVita VT Jr, Hellman S, Rosenberg SA (Eds): Cancer: Principles and Practice of Oncology, 2nd Ed. Philadelphia, Pa, JB Lippincott, 1985, pp 1797-1842

54. Moody TW, Pert CB, Gazdar AF, Carney DN, Minna JD: High levels of intracellular bombesin characterize human small-cell lung carcinoma. Science 1981; 214:1246-1248

55. Sorenson GD, Pettengill OS, Brinck-Johnsen T, Cate CC, Maurer LH: Hormone production by cultures of small-cell carcinoma of the lung. Cancer 1981; 47:1289-1296

56. Sporn MB, Roberts AB: Autocrine growth factors and cancer. Nature 1985; 313:745-747

57. Wood SM, Wood JR, Ghatei MA, Lee YC, O'Shaughnessy D, Bloom SR: Bombesin, somatostatin and neurotensin-like immunoreactivity in bronchial carcinoma. J Clin Endocrinol Metab 1981; 53:1310-1312

58. Yamaguchi K, Abe K, Kameya T, et al: Production and molecular size heterogeneity of immunoreactive gastrin-releasing peptide in fetal and adult lungs and primary lung tumors. Cancer Res 1983; 43:3932-3939

59. Erisman MD, Linnoila RI, Hernandez O, DiAugustine RP, Lazarus LH: Human lung small-cell carcinoma contains bombesin. Proc Natl Acad Sci USA 1982; 79:2379-2383

60. Moody TW, Russell EK, O'Donohue TL, Linden CD, Gazdar AF: Bombesin-like peptides in small cell lung cancer—Biochemical characterization and secretion from a cell line. Life Sci 1983; 32:487-493

61. Korman LY, Carney DN, Citron ML, Moody TW: Secretin/vasoactive intestinal peptide-stimulated secretion of bombesin/gastrin releasing peptide from human small cell carcinoma of the lung. Cancer Res 1986; 46:1214-1218

62. Sorenson GD, Bloom SR, Ghatei MA, Del Prete SA, Cate CC, Pettengill OS: Bombesin production by human small cell carcinoma of the lung. Regul Pept 1982; 4:59-66

63. Moody TW, Bertness V, Carney DN: Bombesin-like peptides and receptors in human tumor cell lines. Peptides 1983; 4:683-686

64. Moody TW, Carney DN, Cuttitta F, Quattrocchi K, Minna JD: High affinity receptors for bombesin/GRP-like peptides on human small cell lung cancer. Life Sci 1985; 37:105-113

65. Carney DN, Cuttitta F, Moody TW, Minna JD: Selective stimulation of small cell lung cancer clonal growth by bombesin and gastrin-releasing peptide. Cancer Res 1987; 47:821-825

66. Trepel JB, Moyer JD, Cuttitta F, et al: A novel bombesin receptor antagonist inhibits autocrine signals in a small cell carcinoma cell line. Biochem Biophys Res Commun 1988; 156:1383-1389

67. Cutz E, Gillan JE, Track NS: Pulmonary endocrine cells in the developing human lung and during neonatal adaptation, *In* Becker KL, Gazdar AF (Eds): The Endocrine Lung in Health and Disease. Philadelphia, Pa, WB Saunders, 1984, pp 210-231

68. Polak JM, Bloom SR: Regulatory peptides: Localization and measurement, *In* Becker KL, Gazdar AF (Eds): The Endocrine Lung in Health and Disease. Philadelphia, Pa, WB Saunders, 1984, pp 300-327

69. Lauweryns JM, Peuskens JC: Neuro-epithelial bodies (neuroreceptor or secretory organs?) in human infant bronchial and bronchiolar epithelium. Anat Rec 1972; 172:471-482

70. Gazdar AF: The biology of endocrine tumors of the lung, *In* Becker KL, Gazdar AF (Eds): The Endocrine Lung in Health and Disease. Philadelphia, Pa, WB Saunders, 1984, pp 448-459

71. Sausville EA, Lebacq-Verheyden AM, Spindel ER, Cuttitta F, Gazdar AF, Battey JF: Expression of the gastrin-releasing peptide gene in human small cell lung cancer: Evidence for alternative processing resulting in three distinct mRNAs. J Biol Chem 1986; 261:2451-2457

72. Lebacq-Verheyden AM, Bertness V, Kirsch I, Hollis GF, McBride OW, Battey J: Human gastrin-releasing peptide gene maps to chromosome band 18q21. Somatic Cell Mol Genet 1987; 13:81-86

73. Cuttitta F, Fedorko J, Gu JA, Lebacq-Verheyden AM, Linnoila RI, Battey JF: Gastrin-releasing peptide gene-associated peptides are expressed in normal human fetal lung and small cell lung cancer: A novel peptide family found in man. J Clin Endocrinol Metab 1988; 67:576-583

74. Willey JC, Lechner JF, Harris CC: Bombesin and the C-terminal tetradecapeptide of gastrin-releasing peptide are growth factors for normal human bronchial epithelial cells. Exp Cell Res 1984; 153:245-248

75. Barnes D, Sato GH: Methods for growth of cultured cells in serum-free medium. Anal Biochem 1980; 102:255-270

76. Jansen M, van Schaik FMA, Ricker AT, et al: Sequence of cDNA encoding human insulin-like growth factor I precursor. Nature 1983; 306:609-611

77. Rotwein P: Two insulin-like growth factor I messenger RNAs are expressed in human liver. Proc Natl Acad Sci USA 1986; 83:77-81

78. Bell GI, Merryweather JP, Sanchez-Pescador R, et al: Sequence of a cDNA clone encoding human preproinsulin-like growth factor II. Nature 1984; 310:775-777

79. Dull TJ, Gray A, Hayflick JS, Ullrich A: Insulin-like growth factor II precursor gene organization in relation to insulin gene family. Nature 1984; 310:777-781

80. Brissenden JE, Ullrich A, Francke U: Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor. Nature 1984; 310:781-784

81. Tricoli JV, Rall LB, Scott J, Bell GI, Shows TB: Localization of insulin-like growth factor genes to human chromosomes 11 and 12. Nature 1984; 310:784-786

82. Jansen M, van Schaik FM, van Tol H, Van den Brande JL, Sussenbach JS: Nucleotide sequences of cDNAs encoding precursors of human insulin-like growth factor II (IGF-II) and an IGF-II variant. FEBS Lett 1985; 179:243-246

83. Rechler MW, Nissley SP: The nature and regulation of the receptors for insulin-like growth factors. Annu Rev Physiol 1985; 47:425-442

84. Froesch ER, Schmid C, Schwander J, Zapf J: Actions of insulin-like growth factors. Annu Rev Physiol 1985; 47:443-467

85. Kull FC Jr, Jacobs S, Su YF, Svoboda ME, Van Wyck JJ, Cuatrecasas P: Monoclonal antibodies to receptors for insulin and somatomedin-C. J Biol Chem 1983; 258:6561-6566

86. Flier JS, Usher P, Moses AC: Monoclonal antibody to the type I insulin-like growth factor (IGF-I) receptor blocks IGF-I receptor-mediated DNA synthesis: Clarification of the mitogenic mechanisms of IGF-I and insulin in human skin fibroblasts. Proc Natl Acad Sci USA 1986; 83:664-668

87. Trowbridge IS, Lopez F: Monoclonal antibody to transferrin receptor blocks transferrin binding and inhibits human cell growth in vitro. Proc Natl Acad Sci USA 1982; 79:1175-1179

88. Mendelsohn J, Trowbridge I, Castagnola J: Inhibition of human lymphocyte proliferation by monoclonal antibody to transferrin receptor. Blood 1983; 62:821-826

89. Lippman ME, Dickson RB, Gelmann EP, et al: Growth regulation of human breast carcinoma occurs through regulated growth factor secretion. J Cell Biochem 1987; 35:1-16

90. Lippman ME, Dickson RB, Gelmann EP, et al: Growth regulatory peptide production by human breast carcinoma cells. J Steroid Biochem 1988; 30:53-61

91. Ervin PR Jr, Kaminski MS, Cody RL, Wicha MS: Production of mammastatin, a tissue-specific growth inhibitor, by normal human mammary cells. Science 1989; 244:1585-1587

92. Salomon DS, Zwiebel JA, Bano M, Losonczy I, Fehnel P, Kidwell WR: Presence of transforming growth factors in human breast cancer cells. Cancer Res 1984; 44:4069-4077

93. Dickson RB, Huff KK, Spencer EM, Lippman ME: Induction of epidermal growth factor-related polypeptides by 17 beta-estradiol in MCF-7 human breast cancer cells. Endocrinology 1986; 118:138-142

94. Bates SE, Davidson NE, Valverius EM, et al: Expression of transforming growth factor alpha and its messenger ribonucleic acid in human breast cancer: Its regulation by estrogen and its possible functional significance. Mol Endocrinol 1988; 2:543-555

95. Fekete M, Wittliff JL, Schally AV: Characteristics and distribution of receptors for [D-TRP6]-luteinizing hormone-releasing hormone, somatostatin, epidermal growth factor, and sex steroids in 500 biopsy samples of human breast cancer. J Clin Lab Anal 1989; 3:137-147

96. Wakeling AE, Newboult E, Peters SW: Effects of antioestrogens on the proliferation of MCF-7 human breast cancer cells. J Mol Endocrinol 1989; 2:225-234

97. Murayama Y, Kurata S, Mishim Y: Regulation of human estrogen receptor gene, epidermal growth factor receptor gene, and oncogenes by estrogen and antiestrogen in MCF-7 breast cancer cells. Cancer Detect Prev 1988; 13:103-107

98. Berthois Y, Dong XF, Martin PM: Regulation of epidermal growth factor-receptor by estrogen and antiestrogen in the human breast cancer cell line MCF-7. Biochem Biophys Res Commun 1989; 159:126-131

99. Kudlow JE, Cheung CYM, Bjorge JD: Epidermal growth factor stimulates the synthesis of its own receptor in a human breast cancer cell line. J Biol Chem 1986; 261:4134-4138

100. Cormier EM, Jordan VC: Contrasting ability of antiestrogens to inhibit MCF-7 growth stimulated by estradiol or epidermal growth factor. Eur J Cancer Clin Oncol 1989; 25:57-63

101. Arteaga CL, Coronado E, Osborne CK: Blockade of the epidermal growth factor receptor inhibits transforming growth factor alpha-induced but not estrogen-induced growth of hormone-dependent human breast cancer. Mol Endocrinol 1988; 2:1064-1069

102. Clarke R, Brünner N, Katz D, et al: The effects of a constitutive expression of transforming growth factor-alpha on the growth of MCF-7 human breast cancer cells in vitro and in vivo. Mol Endocrinol 1989; 3:372-380

103. Karey KP, Sirbasku DA: Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17 beta-estradiol. Cancer Res 1988; 48:4083-4092

104. Peyrat JP, Bonneterre J, Dusanter-Fourt I, Leroy-Martin B, Djiane J, Demaille A: Characterization of insulin-like growth factor 1 receptors (IGF1-R) in human breast cancer cell lines. Bull Cancer (Paris) 1989; 76:311-319

105. De Leon DD, Bakker B, Wilson DM, Hintz RL, Rosenfeld RG: Demonstration of insulin-like growth factor (IGF-I and -II) receptors and binding protein in human breast cancer cell lines. Biochem Biophys Res Commun 1988; 152:398-405

106. Peyrat JP, Bonneterre J, Beuscart R, Djiane J, Demaille A: Insulin-like growth factor 1 receptors in human breast cancer and their relation to estradiol and progesterone receptors. Cancer Res 1988; 48:6429-6433

107. Peyrat JP, Bonneterre J, Laurent JC, et al: Presence and characterization of insulin-like growth factor 1 receptors in human benign breast disease. Eur J Cancer Clin Oncol 1988; 24:1425-1431

108. Pekonen F, Partanen S, Mäkinen T, Rutanen EM: Receptors for epidermal growth factor and insulin-like growth factor I and their relation to steroid receptors in human breast cancer. Cancer Res 1988; 48:1343-1347

109. Huff KK, Knabbe C, Lindsey R, et al: Multihormonal regulation of insulin-like growth factor-I-related protein in MCF-7 human breast cancer cells. Mol Endocrinol 1988; 2:200-208

110. Augereau P, Garcia M, Mattei MG, et al: Cloning and sequencing of the 52K cathepsin D complementary deoxyribonucleic acid of MCF7 breast cancer cells and mapping on chromosome 11. Mol Endocrinol 1988; 2:186-192

111. Capony F, Garcia M, Capdevielle J, Rougeot C, Ferrara P, Rochefort H: Purification and first characterization of the secreted and cellular 52-kDa proteins regulated by estrogens in human-breast cancer cells. Eur J Biochem 1986; 161:505-512

112. Westley B, Rochefort H: A secreted glycoprotein induced by estrogen in human breast cancer cell lines. Cell 1980; 20:353-362

113. Vignon F, Capony F, Chambon M, Freiss G, Garcia M, Rochefort H: Autocrine growth stimulation of the MCF 7 breast cancer cells by the estrogen-regulated 52 K protein. Endocrinology 1986; 118:1537-1545

114. Cavailles V, Garcia M, Rochefort H: Regulation of cathepsin-D and pS2 gene expression by growth factors in MCF7 human breast cancer cells. Mol Endocrinol 1989; 3:552-558

115. Garcia M, Lacombe MJ, Duplay H, et al: Immunohistochemical distribution of the 52-kDa protein in mammary tumors: A marker associated with cell proliferation rather than with hormone responsiveness. J Steroid Biochem 1987; 27:439-445

116. Derynck R, Roberts AB, Winkler ME, et al: Human transforming growth factor-alpha: Precursor structure and expression in E coli. Regul Pept 1988; 22:217-226

117. Gospodarowicz D, Neufeld G, Schweigerer L: Fibroblast growth factor. Mol Cell Endocrinol 1986; 46:187-204

118. Morgan DO, Edman JC, Standring DN, et al: Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 1987; 329:301-307

119. MacDonald RG, Pfeffer SR, Coussens L, et al: A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. Science 1988; 239:1134-1137

120. Capony F, Morisset M, Barrett AJ, et al: Phosphorylation, glycosylation, and proteolytic activity of the 52-kD estrogen-induced protein secreted by MCF7 cells. J Cell Biol 1987; 104:253-262

121. Tally M, Enberg G, Li CH, Hall K: The specificity of the human IGF-2 receptor. Biochem Biophys Res Commun 1987; 147:1206-1212

122. Yee D, Cullen KJ, Paik S, et al: Insulin-like growth factor II mRNA expression in human breast cancer. Cancer Res 1988; 48:6691-6696

123. Theillet C, Le Roy X, De Lapeyrière O, et al: Amplification of FGF-related genes in human tumors: Possible involvement of HST in breast carcinomas. Oncogene 1989; 4:915-922

124. Ali IU, Merlo G, Callahan R, Lidereau R: The amplification unit on chromosome 11q13 in aggressive primary human breast tumors entails the *bcl*-1, *int*-2 and *hst* loci. Oncogene 1989; 4:89-92

125. Takahashi K, Suzuki K, Kawahara S, Ono T: Growth stimulation of human breast epithelial cells by basic fibroblast growth factor in serum-free medium. Int J Cancer 1989; 43:870-874

126. Ro J, Bresser J, Ro JY, Brasfield F, Hortobagyi G, Blick M: SIS/PDGF-B expression in benign and malignant human breast lesions. Oncogene 1989; 4:351-354

127. Eppenberger U: New aspects in the molecular growth regulation of mammary tumors. Recent Results Cancer Res 1989; 113:1-3

128. Peres R, Betsholtz C, Westermark B, Heldin CH: Frequent expression of growth factors for mesenchymal cells in human mammary carcinoma cell lines. Cancer Res 1987; 47:3425-3429

129. Sporn MB, Roberts AB, Wakefield LM, Assoian RK: Transforming growth factor-beta: Biological function and chemical structure. Science 1986; 233:532-534

130. Cheifetz S, Weatherbee JA, Tsang MLS, et al: The transforming growth factor-beta system, a complex pattern of cross-reactive ligands and receptors. Cell 1987; 48:409-415

131. Roberts AB, Anzano MA, Wakefield LM, Roche NS, Stern DF, Sporn MB: Type beta transforming growth factor: A bifunctional regulator of cell growth. Proc Natl Acad Sci USA 1985; 82:119-123

132. Murphy LC, Dotzlaw H: Regulation of transforming growth factor alpha and transforming growth factor beta messenger ribonucleic acid abundance in T-47D, human breast cancer cells. Mol Endocrinol 1989; 3:611-617

133. Cormier EM, Wolf MF, Jordan VC: Decrease in estradiol-stimulated progesterone receptor production in MCF-7 cells by epidermal growth factor and possible clinical implication for paracrine-regulated breast cancer growth. Cancer Res 1989; 49:576-580

134. Arteaga CL, Tandon AK, Von Hoff DD, Osborne CK: Transforming growth factor beta: Potential autocrine growth inhibitor of estrogen receptor-negative human breast cancer cells. Cancer Res 1988; 48:3898-3904

135. Gregory H, Thomas CE, Willshire IR, et al: Epidermal and transforming growth factor alpha in patients with breast tumours. Br J Cancer 1989; 59:605-609

136. Battaglia F, Scambia G, Rossi S, et al: Epidermal growth factor receptor in human breast cancer: Correlation with steroid hormone receptors and axillary lymph node involvement. Eur J Cancer Clin Oncol 1988; 24:1685-1690 [published erratum 1989; 25:577]

137. Toi M, Hamada Y, Nakamura T, et al: Immunocytochemical and biochemical analysis of epidermal growth factor receptor expression in human breast cancer tissues: Relationship to estrogen receptor and lymphatic invasion. Int J Cancer 1989; 43:220-225

138. Nicholson S, Halcrow P, Sainsbury JR, et al: Epidermal growth factor receptor (EGFr) status associated with failure of primary endocrine therapy in elderly postmenopausal patients with breast cancer. Br J Cancer 1988; 58:810-814

139. Nicholson S, Sainsbury JR, Halcrow P, Chambers P, Farndon JR, Harris AL: Expression of epidermal growth factor receptors associated with lack of response to endocrine therapy in recurrent breast cancer. Lancet 1989; 1:182-185

140. Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707-712

141. Zeillinger R, Kury F, Czerwenka K, et al: HER-2 amplification, steroid receptors and epidermal growth factor receptor in primary breast cancer. Oncogene 1989; 4:109-114

142. Hollstein MC, Smits AM, Galiana C, et al: Amplification of epidermal growth factor receptor gene but no evidence of *ras* mutations in primary human esophageal cancers. Cancer Res 1988; 48:5119-5123

143. Ozawa S, Ueda M, Ando N, Abe O, Shimizu N: Epidermal growth factor receptors in cancer tissues of esophagus, lung, pancreas, colorectum, breast and stomach. Jpn J Cancer Res 1988; 79:1201-1207

144. Ochiai A, Takanashi A, Takekura N, et al: Effect of human epidermal growth factor on cell growth and its receptor in human gastric carcinoma cell lines. Jpn J Clin Oncol 1988; 18:15-25

145. Yoshida K, Kyo E, Tsuda T, et al: EGF and TGF-alpha, the ligands of hyperproduced EGFR in human esophageal carcinoma cells, act as autocrine growth factors. Int J Cancer 1990; 45:131-135

146. Bennett C, Paterson IM, Corbishley CM, Luqmani YA: Expression of growth factor and epidermal growth factor receptor encoded transcripts in human gastric tissues. Cancer Res 1989; 49:2104-2111

147. Yasui W, Sumiyoshi H, Hata J, et al: Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. Cancer Res 1988; 48:137-141

148. Pfeiffer A, Rothbauer E, Wiebecke B, Pratschke E, Krämling HJ, Mann K: Increased epidermal growth factor receptors in gastric carcinomas. Gastroenterology 1990; 98:961-967

149. Tahara E, Sumiyoshi H, Hata J, et al: Human epidermal growth factor in gastric carcinoma as a biologic marker of high malignancy. Jpn J Cancer Res 1986; 77:145-152

150. Mori K, Ibaragi S, Kurobe M, Furukawa S, Hayashi K: Production of an hEGF-like immunoreactive factor by human gastric cancer cells depends on differentiational state of the cells. Biochem Biophys Res Commun 1987; 145:1019-1025

151. Yoshida K, Kyo E, Tsujino T, et al: Expression of epidermal growth factor, transforming growth factor-alpha and their receptor genes in human gastric carcinomas: Implication for autocrine growth. Jpn J Cancer Res 1990; 81:43-51

152. Anzano MA, Rieman D, Prichett W, Bowen-Pope DF, Greig R: Growth factor production by human colon carcinoma cell lines. Cancer Res 1989; 49:2898-2904

153. Hanauske AR, Buchok J, Scheithauer W, Von Hoff D: Human colon cancer cell lines secrete alpha TGF-like activity. Br J Cancer 1987; 55:57-59

154. Watkins LF, Brattain MG, Levine AE: Modulation of a high molecular weight form of transforming growth factor-alpha in human colon carcinoma cell lines. Cancer Lett 1988; 40:59-70

155. Yasui W, Hata J, Yokozaki H, et al: Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. Int J Cancer 1988; 41:211-217

156. Rodeck U, Herlyn M, Herlyn D, et al: Tumor growth modulation by a monoclonal antibody to the epidermal growth factor receptor: Immunologically mediated and effector cell-independent effects. Cancer Res 1987; 47:3692-3696

157. Karnes WE Jr, Kim RS, Wu SV, et al: EGF receptor mediates autocrine growth of human colon cancer cell line, SNU-C1 (Abstr). Gastroenterology 1990; 98:A502

158. Hoosein NM, McKnight MK, Levine AE, et al: Differential sensitivity of subclasses of human colon carcinoma cell lines to the growth inhibitory effects of transforming growth factor-beta 1. Exp Cell Res 1989; 181:442-453

159. Hoosein NM, Brattain DE, McKnight MK, Levine AE, Brattain MG: Characterization of the inhibitory effects of transforming growth factor-beta on a human colon carcinoma cell line. Cancer Res 1987; 47:2950-2954

160. Chakrabarty S, Tobon A, Varani J, Brattain MG: Induction of carcinoembryonic antigen secretion and modulation of protein secretion/expression and fibronectin/laminin expression in human colon carcinoma cells by transforming growth factor-beta. Cancer Res 1988; 48:4059-4064

161. Watson S, Durrant L, Morris D: Gastrin: Growth enhancing effects on human gastric and colonic tumour cells. Br J Cancer 1989; 59:554-558

162. Johnson LR: New aspects of the trophic action of gastrointestinal hormones. Gastroenterology 1977; 72:788-792

163. McGregor DB, Morriss LL, Manalo PB, Bomberger RA, Pardini RS: Pentagastrin stimulation of human colon carcinoma. Arch Surg 1989; 124:470-472

164. Watson SA, Durrant LG, Crosbie JD, Morris DL: The in vitro growth re-

sponse of primary human colorectal and gastric cancer cells to gastrin. Int J Cancer 1989; 43:692-696

165. Smith JP, Solomon TE: Effects of gastrin, proglumide, and somatostatin on growth of human colon cancer. Gastroenterology 1988; 95:1541-1548

166. Chicone L, Narayan S, Townsend CM Jr, Singh P: The presence of a 33-40 KDa gastrin binding protein on human and mouse colon cancer. Biochem Biophys Res Commun 1989; 164:512-519

167. Singh P, Rae-Venter B, Townsend CM Jr, Khalil T, Thompson JC: Gastrin receptors in normal and malignant gastrointestinal mucosa: Age-associated changes. Am J Physiol 1985; 249:G761-G769

168. Weinstock J, Baldwin GS: Binding of gastrin(17) to human gastric carcinoma cell lines. Cancer Res 1988; 48:932-937

169. Upp JR Jr, Saydjari R, Townsend CM Jr, Singh P, Barranco SC, Thompson JC: Polyamine levels and gastrin receptors in colon cancers. Ann Surg 1988; 207:662-669

170. Hoosein NM, Kiener PA, Curry RC, Brattain MG: Evidence for autocrine growth stimulation of cultured colon tumor cells by a gastrin/cholecystokinin-like peptide. Exp Cell Res 1990; 186:15-21

171. Hoosein NM, Kiener PA, Curry RC, Rovati LC, McGilbra DK, Brattain MG: Antiproliferative effects of gastrin receptor antagonists and antibodies to gastrin on human colon carcinoma cell lines. Cancer Res 1988; 48:7179-7183

172. Smith JP, Wood JG, Solomon TE: Elevated gastrin levels in patients with colon cancer or adenomatous polyps. Dig Dis Sci 1989; 34:171-174

173. Kadowaki J, Ku N, Oetting WS, Walker AM: Mammotroph autoregulation: Uptake of secreted prolactin and inhibition of secretion. Endocrinology 1984; 114:2060-2067

174. Giss BJ, Walker AM: Mammotroph autoregulation: Intracellular fate of internalized prolactin. Mol Cell Endocrinol 1985; 42:259-267

175. Sinha YN: Structural variants of prolactin, *In* Yoshimura F, Gorbman A (Eds): Pars Distalis of the Pituitary Gland—Structure, Function and Regulation. New York, NY, Elsevier Science, 1986, pp 399-412

176. Nyberg F, Roos P, Wide L: Human pituitary prolactin—Isolation and characterization of three isohormones with different bioassay and radioimmunoassay activities. Biochim Biophys Acta 1980; 625:255-265

177. Ho TWC, Leong FS, Walker AM: Intracellular fluctuations in pituitary prolactin isoform ratios during the rat estrous cycle (Abstr). Endocrinology 1989; 124:403A

178. Tashjian AH Jr: Clonal strains of hormone-producing pituitary cells. Methods Enzymol 1979; 58:527-535

179. Ho TWC, Greenan JR, Walker AM: Mammotroph autoregulation: The differential roles of 24K isoforms of prolactin. Endocrinology 1989; 124:1507-1514

180. Melmed S, Carlson HE, Briggs J, Hershman JM: Autofeedback of prolactin in cultured prolactin-secreting pituitary cells. Horm Res 1980; 12:340-344

181. Oetting WS, Tuazon PT, Traugh JA, Walker AM: Phosphorylation of prolactin. J Biol Chem 1986; 261:1649-1652

182. Greenan JR, Balden E, Ho TWC, Walker AM: Biosynthesis of the secreted 24 K isoforms of prolactin. Endocrinology 1989; 125:2041-2048

183. Oetting WS, Ho TWC, Greenan JR, Walker AM: Production and secretion of the 21-23.5 kDa prolactin-like molecules. Mol Cell Endocrinol 1989; 61:189-199

184. Farquhar MG: Secretion and crinophagy in prolactin cells, *In* Dellmann HD, Johnson JA, Klachko DM (Eds): Comparative Endocrinology of Prolactin. New York, NY, Plenum Press, 1977, pp 37-94

185. Tixier-Vidal A: Anterior pituitary cells in culture, *In* Farquhar MG, Tixier-Vidal A (Eds): Anterior Pituitary. New York, NY, Academic Press, 1975, pp 181-229

186. Greenan JR, Walker AM: Initiation of granulation in a cell line that normally secretes constitutively (Abstr). J Cell Biol 1987; 105:74A

187. Scammell JG, Burrage TG, Dannies PS: Hormonal induction of secretory granules in a pituitary tumor cell line. Endocrinology 1986; 119:1543-1548

188. Ho TWC, Greenan JR, Walker AM: Modulation of prolactin (PRL) isoform and prolactin-like molecular (PLM) storage (Abstr). FASEB J 1988; 2:309

189. Fagin JA, Pixley S, Slanina S, Ong J, Melmed S: Insulin-like growth factor I gene expression in GH3 rat pituitary cells: Messenger ribonucleic acid content, immunocytochemistry and secretion. Endocrinology 1987; 120:2037-2043

190. Johnson TE, Brekhus SD, Ho TWC, et al: Changes in the Prolactin Isoform Profile During DES Prolactinoma Induction. Second International Pituitary Congress, Palm Desert, California, 1989, M-5 (Abstr)