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Mitogenic Regulation of Normal and Malignant Breast Epithelium

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The multiple roles of both estrogenic and polypeptide regulators of mammary epithelial cell growth are reviewed in this article. Effects of both steroidal and peptide hormones are complex and involve multiple interactions with malignant cells and non-malignant host components. Initial carcinogenesis and progression of mammary epithelium to cancer probably require both proliferative stimuli (estrogen, polypeptide growth factors) and genetic damage. This condition may lead to qualitatively different hormonal responses (hormone-responsive cancer). Estrogens can be shown to induce growth-regulatory polypeptide growth factors and interact with them in hormone-dependent breast cancer. Progression of hormone-dependent (estrogen-responsive) breast cancer to hormone independence probably involves multiple mechanisms, including oncogene activation, loss of the estrogen receptor, or loss of hormone responsivity of other gene products. One direction for further therapies may be blockade of hormonal stimulation and interference with necessary activated or induced components of malignant progression such as oncogenes or polypeptide growth factor-receptor systems.

INTRODUCTION

In this review we examine evidence for autocrine and paracrine growth regulation of normal and malignant mammary epithelium. A particular emphasis is placed on the critical role of secreted growth factors and their receptors.

ENDOCRINE, AUTOCRINE, AND PARACRINE MECHANISMS OF GROWTH REGULATION

The development of the complete malignant phenotype depends on interactions between inherited genetic factors, exposure to chemical carcinogens, damaging radiation, oncogenic viruses, and mitogenic hormones, and other promotional agents [1]. Experimental animal model systems [2] have allowed considerable insight into the mechanisms of action of these components; however, the exact etiology of any human cancer has not been fully established. The work of Huggins and Clark [3], linking testicular secretions (androgen) to prostatic carcinoma, and by Beatson [4], linking ovarian secretions (estrogens) to breast carcinoma, represented critical insights into endocrine-dependent neoplasia. In this section we summarize the potential mechanisms of action of systemic estrogen in the human breast cancer process. We also explore the mechanisms of loss of endocrine control of experimental and clinical breast

Abbreviations: EGF: epidermal growth factor FGF: fibroblast growth factor IGF: insulin-like growth factor IL-1: interleukin 1 ODC: ornithine decarboxylase PDGF: platelet-derived growth factor SSV: simian sarcoma virus TGF: transforming growth factor TNF: tumor necrosis factor

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cancer. This process is commonly observed either spontaneously or following the selective pressure of systemic therapy [5].

Loss of estrogenic control of breast cancer growth during malignant progression implies the emergence of other growth controls. Recent work on locally acting, diffusible growth-regulatory substances known as growth factors has provided a model for how additional growth controls might be exerted on mammary epithelial cells ranging from normal epithelium, to hormone-dependent intraductal malignancies, to locally invasive malignancies, to metastatic tumor deposits. Tumors may be accompanied at almost any point by development of resistance to hormonal and chemotherapeutic agents [6]. In some cases, these growth factors can function as autostimulatory or "autocrine" acting substances. In addition, a number of dominantly acting, cancer-inducing genes, known as oncogenes [7,8], have recently been described. Some of these oncogenes code for growth factors or their receptors (e.g., *c-sis*, *c-fms*, *c-erb-b*). Others appear to code for defective, cell membrane-bound growth factor receptors which are enzymatically active even though they lack extracellularly exposed ligand binding sites (e.g., *erb-b*$_2$ or *src*). One oncogene codes for a protein with homology to nuclear receptors for steroid and thyroid hormones and retinoids (*c-erb-a*). Still other oncogenes appear to act distally on growth regulatory pathways, including modulation of transcriptional complexes in the cell nucleus (*fos*, *myc*, and *myb*) or elsewhere in the cell (*ras*) [9,10].

Genetic events which evoke the malignant phenotype probably involve either activation of dominant oncogenes and/or inactivation of dominant cancer-suppressive genes. The mutation of cellular proto-oncogenes (all of which must have some normal function in physiologic growth control or development of normal tissue) to yield highly active (transforming) oncogenes is now known to be extremely important in chemical- and radiation-induced carcinogenesis [1]. Malignant progression of breast cancer, though incompletely defined, probably involves multiple elements; these may include underlying genetic predisposition, mutation, and mitogenesis in response to estrogen, growth factors, and overexpressed growth factor receptors [11,12]. On a cellular level, the actual mechanisms involved in malignant progression remain conjectural. While the entire tumor could undergo progressive, malignant changes, this change is not the most likely scenario. Rather, the bulk of observations suggest that subpopulations of genetically unstable cancer cells continuously arise within the tumor. These additional genetic events result in subpopulations which may have a survival advantage and overtake other, less progressed tumor and normal cells. Surviving subpopulations are modulated by selective pressures: host defenses, competition for nutrients, survival of chemo-hormonal therapeutic agents, and altered environment after metastatic spread [13,14].

Growth control processes in breast cancer are not limited to the malignant cells themselves. Cancer depends upon an intimate and complex interrelationship with non-tumor tissues of the host. The cancer must thwart host immune surveillance and nourish itself as its mass increases [15]. Angiogenesis (blood vessel invasion) and desmoplasia (stromal proliferation), commonly observed surrounding breast cancer, are probably involved [16]. Soluble intercellular mediators of such processes are known as "paracrine" acting hormones. Some of these hormones may also be encoded for by oncogenes such as *c-sis* [17,18]. Development of metastatic potential is reflected by passage across the basement membrane, local invasion, infiltration of blood vessels

and lymphatics, and reseeding in distant metastatic sites. It probably also involves both oncogene- and mitogenic-mediated processes.

Estrogens play an obvious and critical role in normal mammary development. They clearly function both as permissive agents for carcinogen action and as true tumor promotors. Women without functioning ovaries essentially never develop breast cancer, and many breast cancers show an estrogen-dependent or responsive phenotype. A major thesis of this review is that estrogens act by inducing production of locally acting hormones. To identify some of these mediators of growth control, reference may be made to a well-established system for rodent fibroblasts *in vitro*. Smith, Scher, and Todaro, among others, identified "restriction points" in the cell cycle of "normal" (but immortalized) fibroblasts. Various polypeptide growth factors abrogate these restriction points and allow the cell cycle to progress [19]. Platelet-derived growth factor (PDGF) allows cells to pass a restriction point in early G_1. As PDGF acts to initiate the cell cycle, several genes known as proto-oncogenes are sequentially induced. Among these are *c-fos* and *c-myc* in the nucleus, and *c-ras* in the plasma membrane [20]. Epidermal growth factor (EGF) or the related transforming growth factor (TGFa), act later, while insulin-like growth factor I (IGF-I), also known as somatomedin C, and other hormones act still later in G_1. One growth factor may induce another one, which acts further along in the cell cycle. For example, human diploid fibroblasts treated with PDGF, EGF, or growth hormone secrete their own IGF-I. Secreted IGF-I is capable of self-stimulation to promote mitogenesis; anti-IGF-I antibodies block growth hormone stimulation of DNA synthesis.

Fibroblasts and other cells can be transformed with various tumor viruses, oncogenes, chemicals, or radiation. They lose some requirements for exogenous growth factors and produce more of their own as reflected in the decreased serum requirement of some cancer cells [20–23]. Thus, malignant transformation may result from ectopic production of growth factors, which act at restriction points in the cell cycle [24,25].

The ability of some cells to form colonies under anchorage-independent conditions (growth suspended in agar or agarose) is often correlated with their tumorigenicity or state of malignant "transformation" [26]. At least four growth factor activities have been identified which together can reversibly induce this transformed phenotype of murine fibroblasts: PDGF, EGF (or TGFa), IGF-I (or IGF-II, a different somatomedin activity), and an additional growth factor, transforming growth factor β (TGFβ) [25,27,28]. An important aspect of TGFβ's action as a transforming agent appears to be its induction of basement membrane components, such as collagen and fibronectin [29], and of the *c-sis* proto-oncogene [30]. While results using anchorage-independent growth assays suggest that these growth factors are involved in cancer growth control, little direct evidence for an *in vivo* role in tumor growth has yet emerged. In addition, conclusions drawn from the murine fibroblast model system may not apply to other cancers.

The nature of specific restriction point(s) for epithelial cell cycles is unknown. Normal human mammary epithelial cells require a glucocorticoid such as hydrocortisone, insulin, EGF, PGE_1 (a prostaglandin), transferrin, and an incompletely defined pituitary component(s) to proliferate in serum-free medium [31]. In contrast to the fibroblast model, TGFβ is a growth inhibitor for many types of normal and malignant epithelial cells, including breast [32,33]. While some of the same growth factors may facilitate traverse of the cell cycle in both fibroblasts and epithelial cells, control of

anchorage-independent growth may involve another growth factor(s). Halper and Moses [34] have identified an adrenal carcinoma cell line (SW13) which is extremely sensitive for anchorage-independent cloning to a mitogen found in epithelial cancers or cell lines. Basic pituitary fibroblast growth factor (FGF) also stimulates cloning of SW13 cells, and their epithelial cancer-derived growth factor may be a new member of the FGF family. In unpublished studies we have shown that other members of the FGF family (produced occasionally by breast cancer cells) can stimulate clonogenic growth of SW13 cells. Using an independent model system transfected SV40T oncogene, immortalized human mammary epithelial cells, TGFa, EGF, and FGF can also be shown to have transforming activity [35,36]. Finally, in MCF-7 human breast cancer cells, estrogen is capable of inducing anchorage-independent growth [37,38]. Estrogenic control of growth factor activities and elaboration of basement membrane components may contribute to steroid control of the malignant phenotype.

Neoplastic Growth Control

Regulation of neoplastic growth of breast cancer by estrogens is probably a modified remnant of normal regulatory mechanisms for mammary epithelial proliferation and differentiation. Estrogens are mitogens for noth normal and malignant breast epithelium. The hypothalamus-pituitary axis is indirectly in control of ovarian estrogen secretion by virtue of GnRH and gonadotropin stimulation [39]. Whether or not there are direct effects of estrogens on mammary epithelial cells is far less clear. In addition, the pituitary gland (or other organs) may also secrete other direct- or indirect-acting mitogens [40,41] such as IGF-II, FGF, or LHRH. Studies of murine model systems show that estrogen can control breast tumor growth by inducing pituitary synthesis and secretion of prolactin. Sirbasku and colleagues have employed the term "estromedin" for other hypothetical, estrogen-induced mitogens [42]. Estrogen might also act by allowing breast cancers to overcome growth inhibitory agents in their environment or by synergy with other stimulatory agents [43–45]. These interacting components could be serum-derived, produced by the cancer itself, or produced by nearby tissues. Studies of hormonal control of breast cancer have been facilitated by the availability of cancer cell lines, usually derived from pleural or ascites fluids of patients. Several estrogen-responsive lines exist, including MCF-7, T47D, MDA-MB-134, ZR-75-1, PMC42, and CAMA-1 [37,46–61]. The best characterized of these is MCF-7 [46], which has an absolute requirement for estrogen for tumor formation in the athymic (nude) mouse [50]. Experimental findings obtained using cell lines must be regarded with circumspection. After years in laboratory culture, subclonings, and assorted selective pressures, one can only hope that data derived from these cell lines will prove relevant to understanding of tumorigenesis *in vivo*. This hope can only be fulfilled by eventual *in vivo* clinical verification of experimentally derived hypotheses.

Some years ago we and others succeeded in demonstrating receptors for [37,46] and direct proliferative responses to physiologic doses of 17β-estradiol (E_2) *in vitro* [37,48,52,54–59,62] and *in vivo* in the nude mouse [47,50]. A number of other groups failed initially to observe these responses [43–45,49,51]. One problem appears to have been that some groups were working with an incorrectly identified or contaminated MCF-7 cell line [61]. Other discrepancies have now been largely resolved with a more complete understanding of relevant variables in culture conditions. Serum is a rich source of estrogenic compounds, including sulfate conjugates, which must be removed to observe maximal effects of exogenous estrogen *in vitro* [51,54,57]. Furthermore,

phenol red, which is commonly present in culture medium as a pH indicator, can produce estrogenic effects [59,63]. Finally, growth factors, particularly of the insulin family, can critically modulate estrogen responses in the cellular environment [51].

While breast cancer cells may selectively model hormone dependency, their ability to model steps along the pathway toward more malignant behavior is less clear. PMC42, a well-differentiated estrogen-responsive breast cancer cell line, has been recently described. Monoclonal antibodies prepared against surface antigens of this line cross react with intraductal (non-invasive) breast cancer specimens [64,65]. Numerous estrogen-independent breast cancer lines also exist [47]. While existing cell lines can be sorted according to their estrogen receptor status, nearly all were derived from metastatic sites in patients and are fully malignant in that sense. Somewhat confusingly, virtually all are non-metastatic in nude mice. Thus, controls on metastatic behavior have been difficult to address.

We now turn to evidence that estrogens can directly interact with receptor-containing breast cancer cells to modulate gene expression and phenotypic properties. We propose that polypeptide growth factors are common mediators of growth control for normal breast epithelium, estrogen-regulated breast cancer, and autonomous hormone-independent breast cancer. By stressing direct effects of estrogens on cancer cells *in vitro* we do not wish to suggest that growth control of tumors *in vivo* might not be a much more complex phenomenon resulting from interactions among other cell types, hormones, proteases, and basement membrane components.

In isolated cell cultures of clonal lines of human breast cancer cells, estrogens induce a large number of enzymes and other proteins involved in nucleic acid synthesis. These include DNA polymerase, the *c-myc* proto-oncogene [66], thymidine and uridine kinases, thymidylate synthetase, carbamyl phosphate synthetase, aspartate transcarbamylase, dihydroorotase, glucose 6-phosphate dehydrogenase, and dihydrofolate reductase [49,67–69]. Physiologic concentration of estrogen stimulates DNA synthesis by both scavenger and de novo biosynthetic pathways. Estrogen regulates thymidine kinase and dihydrofolate reductase at the mRNA level [70,71]. Regulation of thymidine kinase mRNA occurs at the transcriptional level. Increases in global transcription are tightly coupled to estrogen action [72]. The existence of "second message" regulatory systems in the growth induction process is also possible but has not yet been proven. In MCF-7 cells, estradiol-induced stimulation of phosphatidyl inositol turnover to generate diacylglycerol and inositol-triphosphate occurs with an exceptionally long lag time [73]. In other polypeptide growth factor or protease-induced model systems, this metabolic effect is rapid (within minutes as opposed to hours for estrogenic effects) and tightly coupled to growth control [74,75]. Phosphatidyl inositol turnover is associated with stimulation of Ca^{++} fluxes by inositol-triphosphate and of protein kinase C by Ca^{++} and diacylglycerol. Phosphatidyl inositol could therefore serve as a metabolic mediator of mitogenic effects of estrogen-induced growth factors and/or protease. One potential target for protein kinase C is the Na^+/H^+ antiporter system. The Na^+/H^+ antiporter is activated in a number of mitogen-triggered proliferation systems. Inhibition with the antidiuretic amiloride prevents proliferative responses in some systems [76]. Protein kinase C is not an oncogene; however, its expression can lead to disordered morphology of fibroblasts [77,78].

Ornithine decarboxylase (ODC) is another intracellular target of estrogen action. ODC is covalently linked to cellular membranes through inositol. This bond is broken

by a phosphatidyl inositol-specific phospholipase C, activating the ODC enzyme [79]. ODC activity is associated with induction of proliferation in numerous systems, including breast cancer [80]. The actual contribution to growth control by any of these potential mediators (protein kinase C, ODC, Ca^{++}, or Na^{+}/H^{+} antiporter) remains to be determined.

Progesterone receptor is also induced by estrogen [81]. Estrogen appears to induce the progesterone receptor at the mRNA level [82,83]. Progestins are growth-inhibitory for human breast cancer while inducing a specific protein of 48 kDa [84]. The presence of the progesterone receptor is generally coupled to functional growth regulation by estrogens *in vivo* and *in vitro*. Thus, progesterone receptor content in breast tumors is used as a marker for estrogen and antiestrogen responsiveness of tumors, although exceptions do exist *in vitro* and in some patient tumors [85].

Estrogens and antiestrogens alter the cellular synthesis activity and/or secretion of several other proteins whose role in growth control is unclear. These proteins include various plasminogen activators and collagenolytic enzymes. These proteases may contribute to tumor growth and progression by allowing the tumor to digest and traverse encapsulating basement membrane [86–89]. Proteases may serve additional roles, such as facilitating release of mitogenic growth factors like IGF-I (somatomedin C) from carrier proteins, processing inactive precursor growth factors and proteases to active species [90], or interacting directly with their own cellular receptors [91,92]. In additon, breast cancer cells secrete proteins of 24 kDa [93], 52 and 160 kDa [94,95], 37–39 kDa, 32 kDa [96,97], and 7 kDa (initially identified by detection of an estrogen-induced mRNA species termed pS2) [98,99]. Four other mRNA species, termed pNR 1–4 [100], and the cytoplasmic enzyme LDH [101] are also under estrogen regulation. The 52 kDa glycoprotein, one of the major secreted proteins, has cathepsin D-like activity in purified form; it is also mitogenic for MCF-7 cells *in vitro* [102,103]. A recent study [104] has suggested that secreted cathepsin D-like activity can release and activate cell-associated transforming growth factor alpha (vide infra). The natures of the 160, 37–39, 52, 24, and 7 kDa proteins are unknown at present, but the 160, 52, and 7 kDa secreted proteins may be disassociated from *in vitro* estrogen and antiestrogen modulation of MCF-7 cell growth using two MCF-7 clonal variants [105–107]. These three protein species are decreased by antiestrogen to the same extent in both MCF-7 and LY2, the latter being a stable antiestrogen-resistant variant of MCF-7. In I–13, an MCF-7 clonal variant which is growth-arrested by physiologic concentrations of estrogen, the same three proteins are induced to the same extent as in MCF-7. These observations suggest that (at least *in vitro*) a significant alteration in secretion of these major proteins has no effect on growth in LY2 or I-13.

Estrogen induces the cell surface "receptor" or binding protein for laminin in MCF-7 cells [108,109]. The laminin receptor mediates attachment of cells to basement membrane laminin [88,89] and contributes to invasiveness by tumor cells. Estrogen treatment of MCF-7 cells increases I^{125}-laminin binding, cell attachment to artificial, laminin-coated membranes, and the migration of the same cells across an artificial membrane toward a diffusible source of laminin [109]. Estradiol treatment of MCF-7 cells also induces rearrangements of cytoskeletal and adhesion structures [110] and alterations in the plasma membrane microvilli as observed by scanning electron microscopy [111].

Thus, estrogens exert a considerable number of influences *in vivo* which may indirectly alter breast cancer progression [13]. Direct effects of estrogens on isolated

breast cancer cells are also well established. These effects include growth regulation as well as modulation of enzymes and other activities thought to mediate mitogenic, metastatic, and differentiated status. Some of these activities are secreted normally and can be detected in milk as products of the normal gland [112, 113].

GROWTH FACTOR PRODUCTION BY NORMAL AND MALIGNANT MAMMARY CELLS

We next consider breast cancer-related growth factors and the evidence supporting a pathophysiologic role for them in the growth and malignant progression of mammary epithelium.

Transforming Growth Factor a (TGFa)—Epidermal Growth Factor (EGF) Families

TGFa was initially identified as a secreted product of virally transformed rodent fibroblasts by Delarco, Todaro, Sporn, and Roberts [24,114]. TGFa has subsequently been found in many proliferating normal and malignant human tissues. EGF was initially characterized from rodent salivary glands by Cohen [115] but is now known to be more widely expressed in human tissues. The human form was originally known as urogastrone, a placental product. TGFa activity is known to exist in 25 kDa, 21 kDa, and 17–19 kDa precursor forms [116] and commonly processed to a 7 kDa form. EGF appears to be processed from a very large precursor form (130 kDa) with multiple polypeptide products [117]. EGF, TGFa, and a related protein from vaccinia virus form a functional family of growth factors which apparently utilize the EGF receptor to carry out their many functions [118].

Several lines of evidence show that breast cancer cells produce TGFa. Cell lines secrete stimulatory factors for MCF-7 and murine 3T3 fibroblast monolayer cultures as well as "transforming growth activity" (TGF). This information has been determined by stimulation of anchorage-independent colonies of rodent NRK and AKR-2B fibroblasts in soft agar culture [119–123]. The material produced by some breast cancer cells is a 30 kDa molecular weight species of transforming activity for NRK fibroblasts, which comigrates chromatographically with a peak of MDA-231 autostimulatory activity and is the principal species of EGF receptor-competing activity [121,122]. Antisera specific to TGFa react with this species [124]. Thus, this activity is related to TGFa but appears to be significantly larger than the cloned and sequenced 6 kDa species from transformed rodent fibroblasts [125]. It is not yet certain if this protein is related to the 17–19 kDa TGFa precursor protein observed in transformed fibroblasts [116,126,127], nor is it known if this protein is modified by glycosylation, palmitoylation [116], or if it is the product of alternative mRNA splicing. The precursor species is thought to be membrane bound in cell lines which express it [116,126,127]. It is possible that breast cancer-derived TGFa may be the product of a novel TGFa-related gene. The 30 kDa TGFa-like species is induced by estrogen treatment of estrogen receptor-positive MCF-7, T47D, and ZR-75-1 cells [11,121,122,124,128,129]. It increases by two- to fourteenfold depending on cell type and culture conditions. An expected 4.8 Kb TGFa mRNA species has been detected in MCF-7 and other human breast cancer cell lines and breast tumors [123,130]. This species ranges from low to high estrogen receptor content. No correlation of TGFa mRNA expression was observed with estrogen receptor status; in biopsy samples, at least 70 percent of the adenocarcinomas contained TGFa mRNA [123].

The significance of TGFa induction and secretion has been further examined. TGFa

mRNA is induced in six hours in MCF-7 cells treated with estradiol [123]. Similar observations have been made in hormone-dependent mouse mammary tumors [131]. Using antibodies directed against either TGFa or its receptor (the EGF receptor), we have noted growth suppression of MCF-7 cells grown as anchorage-independent colonies or as estrogen-stimulated, high-density monolayer cultures [123]. Thus, TGFa has a likely role as an autocrine growth factor in experimental breast cancer. Whether this possibility reflects an artifact of adaption of cells to *in vitro* culture conditions must await *in vivo* study. A phase I trial of anti-EGF receptor antibody therapy has been initiated in breast cancer patients [132]. TGFa has also been detected in the urine of patients and nude mice bearing breast and other human tumors [133,134]. Thus it may provide a marker for tumor burden or disease progression. Detection of urinary TGFa has been complicated by the presence of very high levels of EGF-related growth factors present even in normal control urine [133].

Insulin and Insulin-Like Growth Factors and Their Receptors

The insulin family of growth factors is a complex group of cross-reacting ligands, further complicated by multiple receptors, and serum-borne binding proteins. Insulin is a two-chain disulfide-linked growth factor, processed from a single gene product (uncleaved, 7.5 kDa size) whose primary site of synthesis is the pancreas. In contrast to insulin, the single-chain, IGF-I, and IGF-II growth factors (somatomedins) are synthesized by many body tissues (including liver). Somatomedins are under different hormonal regulation, particularly growth hormone [135,136]. Several other growth factors, such as relaxin and lentropin (which controls lens fiber formation) appear to be members of an even larger insulin-related family [137]. Alternative splicing of mRNA of the insulin-like growth factors (particularly IGF-I) [138] further contributes to the complexity of members of this diverse family.

Somatomedins or insulin are required both for anchorage-dependent and independent proliferation of fibroblasts. They may also play a role in breast cancer. IGF-I is mitogenic for some breast cancer cells in culture [139,140]. Using radioimmunoassay, we and others have found that an IGF-I-related species is secreted by all human breast cancer cells examined to date [140,141]. After partial purification from MCF-7 cell-conditioned medium, this growth factor comigrates on gel exclusion chromatography with authentic human serum-derived IGF-I. Acid ethanol extraction is required partially to disrupt a high molecular weight form of the growth factor. A complex series of IGF-I-cross-reacting mRNA species are also detected with Northern blot analysis, using a cDNA probe to authentic IGF-I [140]; however, none of these mRNA species are identifiable as authentic IGF-I upon nuclease protection analysis of mRNA [142]. Complex species of IGF-I cross-hybridizing mRNAs have been previously described for the human fetus [143]. Utilizing phenol red-free medium, there is a three- to sixfold induction of IGF-I-like growth factor with estrogens, TGFa, EGF, or insulin treatment [144]. Secretion of IGF-I-related factors is inhibited by antiestrogens, TGFβ, (in phenol red-containing medium), and glucocorticoids. While growth hormone is a strong stimulus for IGF-I production by liver, fibroblasts, and other normal tissues, it is without effect on production of IGF-I-like growth factors by MCF-7 breast cancer cells [144–148]. IGF-I-related polypeptides are secreted by fibroblasts and smooth muscle and contribute to autocrine growth control in these cell types [146,148,149]. It remains to be seen whether IGF-I produced by breast cancer acts primarily on breast cancer itself in an autostimulatory mode or on surrounding

stroma to promote chemotaxis and growth. Alternatively, breast cancers may induce surrounding mesenchyme to produce IGF activities which function to stimulate the mesenchyme or the breast epithelium. Recent studies have shown that an antibody which blocks the IGF-I receptor [150] is capable of inhibiting MDAMB-231 breast cancer cloning *in vitro* [151] and tumor growth *in vivo* [152]. This finding suggests that importance of an autocrine or paracrine role for growth factors acting through the IGF-I receptor.

Since insulin synergizes with estrogen in promoting growth of breast cancer cell lines *in vitro* and *in vivo* in the nude mouse, it is possible that somatomedins principally act by interacting with estrogen to promote breast tumor growth in hormone-dependent cells; their role in hormone-independent cells is even more obscure [153,154].

It has also been reported that IGF-II-related gene product(s) are produced by normal and malignant tissue [155]. IGF-II appears to bind to multiple receptors (insulin, IGF-II, and IGF-I). All of these receptors, including the IGF-II receptor, have been detected in human breast cancer [139,156] as well as in normal breast tissue [157]. While IGF-II interaction with IGF-I receptors may stimulate cellular response, IGF-II receptors may be primarily involved in IGF-II degradation. The IGF-II receptor is a multifunctional protein, previously described as the mannose-6-phosphate receptor for lysosomal enzymes [158,159]. IGF-I-like mRNA has been recently reported in other human tumors: lung, colon, and liposarcoma [160–162]. IGF-II has been observed to be overproduced in Wilms tumor [163]. Somatomedins appear to be among the most ubiquitous growth factors, produced by nearly all normal tissues [164–166] and found in the blood [167] and urine [168].

Transforming Growth Factor β (TGFβ)

Transforming growth factor beta (TGFβ) is a 25 kDa homodimer initially purified from platelets and various normal tissues. It is required (along with other growth factors) for full induction of the transformed phenotype in fibroblasts. It is also produced autonomously in fibroblasts transformed by oncogenes [169]. TGFβ is a member of a multi-gene family which includes four TGFβ's, Mullerian inhibiting substance, inhibins and activins [170], a T-cell suppressor factor [171], and a Drosophila morphogenesis-controlling gene known as decapentaplegic [172]. In contrast to TGFa and many other growth factors, $TGF\beta_1$ is growth-inhibitory and/or differentiating-promoting for most epithelial cells [32,33,173]. For example, it inhibits myogenic differentiation [174,175]. It also inhibits normal hepatocyte growth more extensively than malignant liver cell growth, and it prevents dedifferentiation of other epithelial cell types [176,177]. In addition, it stimulates differentiated behavior of vascular smooth muscle and normal breast epithelial cultures [178].

Normal mammary epithelial cells are induced by $TGF\beta_1$ or $TGFb_2$ to synthesize milk fat globule antigen. In addition, growth of these cells in culture is arrested and the morphology is markedly altered; $TGF\beta_1$ or $TGFb_2$ changes the cobblestone epithelial appearance to an elongated spindle shape [178]. TGFβ also appears to be extremely potent *in vivo* in the neonatal mouse. Implants of TGFβ in slow-release capsules near developing mammary ducts result in complete cessation of mammary ductal development. No effects of TGFβ are seen on surrounding stromal tissue or on more distant mammary glands [179]. TGFβ may play a role, along with other hormones such as estrogen and growth factors (such as EGF or TGFa) [180], in the delicately balanced

process of human mammary development. TGFβ (along with a plethora of other growth factors) is also a component of human milk [181].

Breast cancer, like normal breast epithelium, is inhibited by $TGF\beta_1$ or $TGFb_2$. TGFβ may be an autocrine-inhibitory type of substance (chalone) [182] in breast cancer. Breast cancer cells have been shown to contain and secrete a TGFβ-related activity [33,121,183–185]. The activity binds to TGFβ receptors, transforms AKR-2B and NRK fibroblasts, is immunoprecipitated by TGF-beta antiserum, and comigrates on gel exclusion columns with platelet-derived TGFβ [184]. All breast cancer cell lines reported express the expected 2.5 Kb mRNA species [182,184,186]. $TGF\beta_1$ secretion is inhibited by treatment of MCF-7 cells with estrogen and insulin [184], but growth-inhibitory antiestrogens and glucocorticoids strongly stimulate its secretion. Intracellular $TGF\beta_1$ does not appear to change in concentration following treatment with mitogens or growth inhibitors [184]. TGFβ activity from antiestrogen-induced MCF-7 cells strongly inhibits the growth of an estrogen receptor-negative cell line MDA-MB-231. This growth inhibition was partially reversed in the presence of a polyclonal antibody directed against native $TGF\beta_1$ [184]. Since breast cancers exist as mixtures of estrogen receptor-positive and negative tumor cells [187,188] and breast cancers may not become TGFβ-unresponsive as they become antiestrogen-unresponsive, TGFβ may act in tumors with such mixed cell populations to make antiestrogen more effective than might otherwise be expected [184]. In LY2 cells, an MCF-7 variant stepwise selected *in vitro* for antiestrogen resistance, TGFβ is no longer induced by antiestrogen, but the cells still retain the TGFβ receptor and response. Neither the mechanism of $TGF\beta_1$ induction in MCF-7 cells nor its loss in LY2 cells is fully defined, but it is not at the regulation of steady-state mRNA level. Conversion of a latent form to an active form of $TGF\beta_1$ may contribute [184]. In contrast to other cell types [168,189], there is significant active TGFβ present in breast cancer-conditioned medium. The biochemical details of the conversion of a secreted inactive to active TGFβ remain to be elucidated.

Platelet-Derived Growth Factor (PDGF)

PDGF is a heterodimeric protein of approximately 30 kDa, which, as the name implies, is found in high concentrations in platelets. PDGF-like related growth factors are also produced by a variety of transformed murine fibroblast lines and by some human tumors of diverse origins. The *v-sis* oncogene is related to a PDGF B chain homodimer and can transform PDGF-receptor-expressing cell types [20]. Consequently, PDGF could fulfill an autocrine role in such tumors. In tumors derived from cell types lacking the PDGF receptor, *v-sis* is not transforming and PDGF presumably functions in a paracrine mode. Paracrine action may stimulate angiogenesis, stromal proliferation (desmoplasia), and chemotaxis and degranulation of monocytes and neutrophils [190].

Simian sarcoma virus- (SSV) transformed fibroblastic cells provide a model system for the function of PDGF in responsive cell types. In this system, the PDGF-B chain-related protein encoded by the virus forms a homodimer and is sometimes secreted by the cell. Antibodies directed against PDGF have been reported to exert antiproliferative and antitransforming activity [190]; however, in many instances the PDGF is largely cell-associated and presumably already bound to its receptor. Thus, anti-PDGF antisera have been only partially effective as antiproliferative reagents [191]. The subcellular origin or fate of PDGF remains to be fully characterized;

however, immunoreactive PDGF has been observed in the cell nucleus [192] in SSV-transformed cells. PDGF is known to encode a short peptide sequence which is capable of directing the molecule across the nuclear pore complex [193]. Thus, PDGF may exert autocrine growth control through both intracellular and extracellular mechanisms of action [194].

Many breast cancer cell lines secrete a PDGF-related activity detected by anchorage-dependent growth stimulation of mouse 3T3 fibroblasts in the presence of platelet-poor plasma. This fact is known as a "competency" assay for early mitogenic signals [195]. 28 kDa and 16 kDa species were observed by immunoprecipitation of metabolically labeled MCF-7, MDA-MB-231, and other breast cancer cell extracts and medium. The 28 kDa species (the unreduced form) was biologically active after elution from non-reducing SDS-polyacrylamide gels, and its activity was blocked with anti-PDGF antiserum. Upon examination of poly A-selected mRNA from either cell line, transcripts of both PDGF-A and B chains are observed [196–199]. A and B chains are widely expressed in breast cancer and other cell lines [200–203]. While the B chain is homologous to the *v-sis* oncogene, the A chain is not known to have a retroviral oncogene homologue. The A and B chains share substantial sequence homology to each other [204], and the A chain shows evidence of alternative mRNA splicing [205]. It is not yet known how A and B chains assemble in breast cancer cells.

The Fibroblast Growth Factors (FGF)

The fibroblast growth factors, like PDGF, were initially classified as "competency" factors acting early in the G_1 phase of the cell cycle to stimulate the growth of mesenchymal cells. The members of the family include acidic and basic FGF [206] (aFGF and bFGF), kaposi FGF (kFGF, also known as *hst,* for human stomach tumor oncogene) [207], *int 2* [208] (a mouse mammary cancer oncogene mentioned earlier), FGF-5 [209], and others less well characterized. A more distant homology also exists with interleukin 1 (IL-1) [210]. It is not yet known how many classes of receptors exist for this diverse class of ligands. Both aFGF and bFGF bind a 140–210 kDa receptor and stimulate tyrosine phosphorylation of a 90 kDa protein [211]. An FGF receptor has recently been purified to apparent homogeneity. bFGF is capable of acting as an oncogene when expressed in fibroblasts in association with some means of secretion, and secreted members of the family such as LST and FGF-5 can function as bona fide oncogenes [212,213].

FGF is required for normal mouse mammary cells to proliferate in culture. It is present in pituitary extract used for culture of mouse and human mammary myoepithelial and epithelial cells [214,215]. FGF is also a potent angiogenic substance [210]. The FGF family of peptides is characterized by a binding site for heparin [206]. This property has facilitated purification and may allow for strategies to interrupt or otherwise modulate FGF action through binding of various polyanionic substances such as suramin to this site [216]. bFGF does not possess a signal peptide in its primary sequence [217], giving rise to hypotheses for unusual secretory pathways, including intracellular binding to heparin proteoglycan and secretion as a part of the basement membrane [206].

Uncertainty exists as to the principal target of FGF in the normal mammary gland. Rudland has proposed that effects are restricted to myoepithelium and stroma [218], but Karey and Sirbasku have claimed that MCF-7 and T47D human breast carcinoma

cells respond [219]. We have not been able to confirm this result. A 60 kDa, heparin-binding FGF-related molecule is produced by human breast cancer cells in culture. Its function could include autocrine and paracrine effects [220].

Pituitary Hormones, Other Steroids, and Growth Factors

Growth factors and/or estrogen probably act in concert with other systemic mitogens *in vivo* to promote tumor growth. Shiu and co-workers have isolated a pituitary-derived activity which potentiates the mitogenic effects of estradiol on MCF-7 cells [221]. One pituitary factor has already been identified as IGF-II [222]. In addition, pituitary-derived GnRH may also directly interact with breast cancer to inhibit its proliferation [223], while prolactin is stimulatory for some cell lines [40].

MCF-7 cells in monolayer culture are growth-regulated by a variety of lipid-soluble hormones in addition to estrogen. These hormones include glucocorticoids, iodothyronines, androgens, and retinoids [224]. MCF-7 cells have receptors but are not growth-stimulated by progesterone or vitamin D [225–227]. Progesterone induces a specific protein [226] and can be growth-inhibitory *in vitro* [228]. Other inhibitory hormones include somatostatin [229], interleukins 1 and 6, tumor necrosis factor (TNF), and interferon [230]. Receptors and metabolic effects, but little cellular growth response, have been demonstrated for other hormones, such as growth hormone, glucagon, and calcitonin [227]. Finally, transferrin, a serum iron delivery molecule, is required for proliferation of normal and malignant mammary cells [231]; its receptor is increased in estrogen-independent breast cancer compared to estrogen-dependent breast cancer [232].

The multiplicity of growth modulatory hormones for *in vitro* breast cancer systems suggests the possibility that many serum-borne or locally produced modulators of growth may play important regulatory roles *in vivo*. Alternatively, or additionally, growth factors with a similar spectrum of activities could be elaborated by the breast cancer cells themselves. While on the one hand, this large number of regulatory molecules emphasizes the potential complexity of growth control of breast cancer, it can also be seen as emphasizing the diverse number of targets for biological therapy. A variety of strategies including anti-ligand and anti-receptor antibodies, receptor-blocking peptides, drugs which alter receptor ligand interaction, and anti-sense RNA strategies all have promise for novel approaches to the problem of breast cancer.

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REFERENCES

1. Bishop JM: The molecular genetics of cancer. Science 235:305–311, 1987
2. Welsch CW: Rodent models to examine *in vivo* hormonal regulation of mammary gland tumorigenesis. In Cellular and Molecular Biology of Mammary Cancer. Edited by D Medina, W Kidwell, G Heppner, E Anderson. New York, Plenum Press, 1987, pp 153–179
3. Huggins C, Clark PJ: Quantitative studies of prostatic secretion. J Exp Med 72:747–762, 1940
4. Beatson GT: On the treatment of inoperable cases of carcinoma of the mamma; suggestions for a new method of treatment with illustrative cases. Lancet ii:104–107, 162–165, 1896
5. Lippman ME: Efforts to combine endocrine and chemotherapy in the management of breast cancer: Do two and two equal three? Breast Cancer Res Treat 3:117–127, 1983
6. Dickson RB, Lippman ME: Estrogen regulation of growth and polypeptide growth factor secretion in human breast carcinoma. Endocrine Rev 8:29–43, 1986
7. Pardee AD: Molecules involved in proliferation of normal and cancer cells. Cancer Res 47:1488–1491, 1987
8. Slamon DJ, DeKernion JB, Verma IM, Cline MJ: Expression of cellular oncogenes in human malignancies. Science 224:256–262, 1984
9. Studzinski GP, Brelvi ZS, Feldman SC, Watt RA: Participation of c-myc protein in DNA synthesis of human cells. Science 234:467–470, 1986
10. Bohmann D, Bos TJ, Admon A, Nishimura T, Vogt PK, Tjian R: Human protooncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science 238:1386–1392, 1987
11. Dickson RB, Rosen N, Gelmann EP, Lippman ME: Receptor and signal transduction-related protooncogenes in breast cancer. Trends in Pharmacological Science 8:372–375, 1987
12. Cline MJ, Buttitora H, Yokota J: Protooncogene abnormalities in human breast cancer: Correlations with anatomic features and clinical course of disease. J Clin Oncol 5:999–1006, 1987
13. Nowell PC: Mechanisms of tumor progression. Cancer Res 46:2203–2207, 1986
14. Nicholson GL: Tumor cell instability, diversification, and progression to the metastatic phenotype: From oncogene to oncofetal expression. Cancer Res 47:1473–1479, 1987
15. Prehn RT, Prehn LM: The autoimmune nature of cancer. Cancer Res 47:927–932, 1987
16. Folkman J: How is blood vessel growth regulated in normal and neoplastic tissue. Cancer Res 46:467–473, 1986
17. Sporn MB, Roberts AB, Driscoll JS: Principles of cancer biology growth factors and differentiation. In Cancer: Principles and Practice of Oncology, 2nd edition. Edited by VT DeVita, S Hellman, SA Rosenberg. Philadelphia, JB Lippincott Co, 1985, pp 49–65
18. Sporn MB, Roberts AB: Autocrine, paracrine and endocrine mechanisms of growth control. Cancer Surveys 4:627–632, 1985
19. Smith HS, Scher CD, Todaro GJ: Induction of cell division in medium lacking serum growth factor by SV40. Virology 44:359–370, 1971
20. Heldin CH, Westermark B: Growth factors: Mechanism of action and relations to oncogenes. Cell 37:9–20, 1984
21. Cherington PV, Smith BL, Pardee AB: Loss of epidermal growth factor requirement and malignant transformation. Proc Natl Acad Sci USA 76:3937–3942, 1979
22. Bradshaw GL, Dubes GR: Polyoma virus transformation of rat kidney fibroblasts results in loss of requirement for insulin and retinoic acid. J Gen Virol 64:2311–2315, 1984
23. Zhan X, Goldfarb M: Growth factor requirements of oncogene-transformed NIH 3T3 and Balb/c 3T3 cells cultured in defined media. Molecular and Cellular Biology 6:3541–3544, 1986
24. Delarco JE, Todaro GJ: Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA 75:4001–4005, 1978
25. Sporn MB, Todaro GJ: Autocrine secretion and malignant transformation of cells. N Engl J Med 303:878–880, 1980
26. Freedman VH, Shiu S: Cellular tumorigenicity in nude mice: Correlation with cell growth in semi-solid medium. Cell 3:355–359, 1974
27. Assoian RK, Grotendorst GR, Miller DM, Sporn MB: Cellular transformation by coordinated action of three peptide growth factors from human platelets. Nature (London) 309:804–806, 1984
28. Massague J, Kelly B, Mottola C: Stimulation by insulin-like growth factors is required for cellular transformation by type transforming growth factor. J Biol Chem 260:4551–4554, 1985

29. Ignotz RA, Massague J: Transforming growth factor β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261:4337–4345, 1986
30. Leof EB, Proper JA, Goustin AJ, Shipley GD, Dicorleto PE, Moses HL: Induction of c-sis mRNA and activity similar to platelet-derived growth factor: A proposed model for indirect mitogenesis involving autocrine activity. Proc Natl Acad Sci USA 83:2453–2457, 1986
31. Hammond SL, Ham RG, Stampfer MR: Serum-free growth of human mammary epithelial cells: Rapid clonal growth in defined medium and extended serial passage with pituitary extract. Proc Natl Sci USA 81:5435–5439, 1984
32. Tucker RF, Shipley GD, Moses HL, Holley RW: Growth inhibitor from BSC-1 cells closely related to platelet type β transforming growth factor. Science 226:705–707, 1984
33. Roberts AB, Anzano MA, Wakefield LM, Roche NS, Stern DF, Sporn MB: Type β transforming growth factor: A bifunctional regulator of cellular growth. Proc Natl Acad Sci USA 82:119–123, 1985
34. Halper J, Moses HL: Epithelial tissue-derived growth factor-like polypeptides. Cancer Res 43:1972–1979, 1983
35. Clark R, Stampfer MR, Milley R, O'Rourke E, Walen KH, Kriegler M, Kopplin J, McCormick F: Transformation of human mammary epithelial cells by oncogenic retroviruses. Cancer Res, in press
36. Valverius E, Bates S, Stampfer M, Clarke R, McCormick F, Salomon D, Lippman ME, Dickson RB: Transforming growth factor alpha production and EGF receptor expression in human mammary epithelial cells: Oncogenic influence on function EGR of receptor. Molecular Endocrinology, in press
37. Lippman ME, Bolan G, Huff K: The effects of estrogens and antiestrogens on hormone-response human breast cancer in long term tissue culture. Cancer Res 36:4595–4601, 1976
38. Sutherland RL, Reddel RR, Murphy LC, Taylor IW: Effects of antiestrogens on cell cycle progression. In Estrogen/Antiestrogen Action and Breast Cancer Therapy. Edited by JC Jordan. Madison WI, University of Wisconsin Press, 1986, pp 93–114
39. Ross GT, Vande Wiele RL, Frantz AG: The ovaries and the breasts. In Textbook of Endocrinology. Edited by RH Williams. Philadelphia, WB Saunders Co, 1981, pp 355–411
40. Eidne KA, Flanagan CA, Millar RP: Gonadotropin-releasing hormone binding sites in human breast carcinoma. Science 229:989–991, 1985
41. Wilding G, Chen M, Gelmann EP, Miller WR: LHRH agonists and human breast cancer cells. Nature 329:770, 1987
42. Ikeda T, Danielpour D, Sirbasku BA: Isolation and properties of endocrine and autocrine type mammary tumor cell growth factors (estromedins). In Progress in Cancer Research and Therapy, Volume 31. Edited by F Bresciani, RJB King, ME Lippman, M Namer, JP Raynaud. New York, Raven Press, 1983, pp 171–186
43. Lykkesfeldt AE, Briand P: Indirect mechanism of estradiol stimulation of cell proliferation of human breast cancer cell lines. Br J Cancer 53:29–35, 1986
44. Devleeschouwer N, Legros N, Oleu-Serrano N, Pariduens R, Leclerq G: Estrogen conjugates and serum factors mediating the estrogenic trophic effect on MCF-7 cell growth. Cancer Res 47:5883–5887, 1987
45. Soto A, Sonnenschein C: Cell proliferation of estrogen-sensitive cells: The case for negative control. Endocrine Rev 8:44–52, 1987
46. Brooks SC, Locke ER, Soule HD: Estrogen receptor in a human breast cell line (MCF-7) from breast carcinoma. J Biol Chem 248:6251–6261, 1973
47. Engle LW, Young NW: Human breast carcinoma cells in continuous culture: A review: Cancer Res 38:4327–4339, 1978
48. Weichselbaum RW, Hellman S, Piro A, Nove JJ, Little JB: Proliferation kinetics of a human breast cancer cell line in vitro following treatment with 17β-estradiol and 1-B-o-arabinofuranasylcytosine. Cancer Res 38:2339–2342, 1978
49. Edwards DP, Murphy SR, McGuire WL: Effect of estrogen and antiestrogen on DNA polymerase in human breast cancer. Cancer Res 40:1722–1726, 1980
50. Soule HD, McGrath CM: Estrogen responsive proliferation of clonal human breast carcinoma cells in athymic mice. Cancer Lett 10:177–189, 1980
51. Butler WB, Kelsey WH, Goran N: Effects of serum and insulin on the sensitivity of the human breast cancer cell line MCF-7 to estrogens and antiestrogens. Cancer Res 41:82–88, 1981
52. Chablos D, Vignon F, Keydar I, Rochefort H: Estrogens stimulate cell proliferation and induce secretory proteins in a human breast cancer cell line (T47D). J Clin Endocrinol Metab 55:276–283, 1982

53. Leung BS, Qureshi S, Leung JS: Response to estrogen by the human mammary carcinoma cell line CAMA-1. Cancer Res 42:5060–5066, 1982
54. Darbre P, Yates J, Curtis S, King RJB: Effect of estradiol on human breast cancer cells in culture. Cancer Res 43:349–354, 1983
55. Katzenellenbogen BS, Norman MJ, Eckert RL, Peltz SW, Mangel WF: Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxytamoxifen isomers in MCF-7 human breast cancer cells. Cancer Res 44:112–119, 1983
56. Natoli C, Sica G, Natoli V, Serra A, Iacobelli S: Two new estrogen-supersensitive variants of the MCF-7 human breast cancer cell line. Breast Cancer Res Treat 3:23–32, 1983
57. Page MJ, Field JK, Everett NP, Green CD: Serum-regulation of the estrogen responsiveness of the human breast cancer cell line MCF-7. Cancer Res 43:1244–1249, 1983
58. Simon WE, Albrecht M, Trams G, Dietel M, Holzer F: In vitro growth promotion of human mammary carcinoma cells by steroid hormones, tamoxifen, and prolactin. JNCI 73:313–321, 1984
59. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS: Phenol red in tissue culture media is a weak estrogen: Implications concerning the study of estrogen-responsive cells in culture. Proc Natl Acad Sci USA 83:2496–2500, 1986
60. Reiner GCA, Katzenellenbogen BS: Characterization of estrogen receptor in MDA-MB-134 human breast cancer cells. Cancer Res 46:1124–1131, 1986
61. Osborne CK, Hobbs K, Trent JM: Biological differences among MCF-7 human breast cancer cell lines from different laboratories. Breast Cancer Res Treat 9:111–121, 1987
62. Darbre P, Yates J, Curtis S, King RJB: Effect of estradiol on human breast cancer cells in culture. Cancer Res 43:349–354, 1983
63. Nelson JN, Clarke R, McFerran NV, Murphy RF: Morpho-functional effects of phenol red on oestrogen-sensitive breast cancer cells. Biochemical Society Trans 15:244, 1987
64. Whitehead RH, Quirk SJ, Vitali AA, et al: A new human breast carcinoma cell line (PMC42) with stem cell characteristics. III. Hormone receptor status and responsiveness. JNCI 73:643–648, 1984
65. Dempsey PJ, Feleppa FP, Brown RW, deKretser TA, Whitehead RH, Jose DG: Development of monoclonal antibodies to the human breast carcinoma cell line PMC42. JNCI 77:1–15, 1986
66. Dubik D, Demboinsiki TC, Shiu RP: Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells. Cancer Res 47:6517–6521, 1987
67. Aitken SC, Lippman ME: Hormonal regulation of de novo pyrimidine synthesis and utilization in human breast cancer cells in tissue culture. Cancer Res 43:4681–4690, 1983
68. Aitken SC, Lippman ME: Effect of estrogens and antiestrogens on growth-regulatory enzymes in human breast cancer cells in tissue culture. Cancer Res 45:1611–1620, 1985
69. Dickson RB, Aitken S, Lippman ME: Assay of mitogen-induced effects on cellular thymidine incorporation. In Methods in Enzymology 46: Hormone Action Part II: Peptide Growth Factors. Edited by D Barnes, DA Sirbasku. New York, Academic Press, 1987, pp 327–340
70. Cowan K, Levine R, Aitken S, Goldsmith M, Douglass E, Clendeninn N, Nienhuis A, Lippman ME: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexate resistant human breast cancer cells. J Biol Chem 257:15079–15086, 1982
71. Kasid A, Davidson N, Gelmann E, Lippman ME: Transcriptional control of thymidine kinase gene expression by estrogens and antiestrogens in MCF-7 human breast cancer cells. J Biol Chem 261:5562–5567, 1986
72. Aitken SC, Lippman ME, Kasid A, Schoenberg DR: Relationship between the expression of estrogen regulated genes and estrogen-stimulated proliferation of MCF-7 mammary tumor cells. Cancer Res 45:2608-2615, 1985
73. Freter CE, Lippman ME, Cheville A, Zinn S: Alterations in phosphoinositide metabolism associated with 17B-estradial and growth factor treatment of MCF-7 breast cancer cells. Molecular Endocrinology 2:159–166, 1988
74. Nishizuka Y: Protein kinases in signal transduction. Trends Biochem Sci 9:163–171, 1984
75. Carney DH, Scott DL, Gordon EA, LaBelle EF: Phosphoinositides in mitogenesis: Neomycin inhibits thrombin-stimulated phosphoinositide turnover and initiation of cell proliferation. Cell 42:479–488, 1985
76. Davis S, Pouyssegur J: Pertussis toxin inhibits thrombin-induced activation of phosphoinositide hydrolysis and Na^+/H^+ exchange in hamster fibroblasts. EMBO J 5:55–60, 1986
77. Housley GM, Johnson MD, Wendy-Hsiao WL, O'Brian CA, Murphy JP, Kirschmeier P, Weinstein IB: Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. Cell 52:343–354, 1988

78. Persons DA, Wilkerson WO, Bell RM, Finn OJ: Altered growth regulation and enhanced tumorigenicity of NIH 3T3 fibroblasts transfected with protein kinase C-1 cDNA. Cell 52:447–458, 1988
79. Mustelin T, Poso H, Lapinjoki SP, Gynther J, Andersson LC: Growth signal transduction: Rapid activation of covalently bound ornithine decarboxylase during phosphatidylinositol breakdown. Cell 49:171–176, 1987
80. Manni A, Wright C, Feil P, Baranao L, Demers L, Garcia M, Rochefort H: Autocrine stimulation by estradiol-regulated growth factor of rat hormone-responsive mammary cancer: Interaction with the polyamine pathway. Cancer Res 46:1594–1600, 1986
81. Horwitz KB, McGuire WL: Estrogen control of progesterone receptor in human breast cancer. J Biol Chem 253:2223–2228, 1978
82. Nardulli AM, Greene GL, O'Malley BW, Katzenellenbogen BS: Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF-7 cells by estradiol: Analysis of estrogen's effect on progesterone receptor synthesis and degradation. Endocrinology 122:935–944, 1988
83. Read LD, Snider CE, Miller JS, Greene GL, Katzenellenbogen BS: Ligand modulated regulation of progesterone receptor messenger ribonucleic acid and protein in human breast cancer cell lines. Mol Endocrinol 2:263–271, 1988
84. Chablos D, Rochefort H: Dual effects of progestin R5020 on proteins released by the T47D human breast cancer cells. J Biol Chem 259:1231–1238, 1984
85. Lippman ME: Endocrine responsive cancers of man. In Textbook of Endocrinology. Edited by RH Williams. Philadelphia, WB Saunders Co, 1985, pp 1309–1326
86. Butler WB, Kirkland WL, Jorgensen TL: Induction of plasminogen activator by estrogen in a human breast cancer cell line (MCF-7). Biochem Biophys Res Comm 90:1328–1334, 1979
87. Huff KK, Lippman ME: Hormonal control of plasminogen activator secretion in ZR-75-1 human breast cancer cells in culture. Endocrinology 114:1665–1671, 1984
88. Liotta LA, Rao CN, Weiner UM: Biochemical interactions of tumor cells with the basement membrane. Ann Rev Biochem 55:1037–1057, 1986
89. Terranova VP, Hujanen ES, Martin GR: Basement membrane and the invasive activity of metastatic tumor cells. JNCI 77:311–316, 1986
90. Kaufman U, Zapf J, Torretti B, Froesch ER: Demonstration of a specific serum carrier protein of nonsuppressible insulin-like activity in vivo. J Clin Endocrinol Metab 44:160–166, 1977
91. Neufeld EF, Ashwell G: In The Biochemistry of Glycoproteins and Proteoglycans. Edited by WJ Lennarz. New York, Plenum Press, 1980, pp 241–266
92. Stoppelli MP, Tacchetti C, Cubellis MV, Corti A, Hearing VJ, Cassani G, Appella E, Blasi F: Autocrine saturation of pro-urokinase receptors on human A431 cells. Cell 45:675–684, 1986
93. Ciocca DR, Adams DJ, Edwards DP, Bjerke RJ, McGuire WL: Distribution of an estrogen induced protein with a molecular weight of 24,000 in normal and malignant human tissues and cells. Cancer Res 43:1204–1210, 1983
94. Westley B, Rochefort H: A secreted glycoprotein induced by estrogen in human breast cancer cell lines. Cell 20:353–362, 1980
95. Westley BR, May FEB: Oestrogen regulates cathepsin D mRNA levels in oestrogen responsive human breast cancer cells. Nucleic Acids Res 15:3773–3780, 1987
96. Bronzert DA, Silverman S, Lippman ME: Estrogen inhibition of a Mr 39,000 glycoprotein secreted by human breast cancer cells. Cancer Res 47:1234–1238, 1987
97. Sheen YY, Katzenellenbogen BS: Antiestrogen stimulation of the production of a 37,000 molecular weight secreted protein and estrogen stimulation of the production of a 32,000 molecular weight secreted protein in MCF-7 human breast cancer cells. Endocrinology 120:1140–1151, 1987
98. Jakolew SB, Breathneck R, Jeltsch J, Chambon P: Sequence of the pS2 mRNA induced estrogen in the human breast cancer cell line MCF-7. Nucleic Acids Res 12:2861–2874, 1984
99. Nunez AM, Jakolew S, Briand JP, Gaire M, Krust A, Rio MC, Chambon P: Characterization of the estrogen-induced pS2 protein secreted by the human breast cancer cell line MCF-7. Endocrinology 121:1758–1765, 1987
100. May FEB, Westley BR: Cloning of estrogen-regulated messenger RNA sequences from human breast cancer cells. Cancer Res 46:6034–6040, 1986
101. Burke RE, Harris SC, McGuire WL: Lactate dehydrogenase in estrogen responsive human breast cancer cells. Cancer Res 38:2773–2780, 1978
102. Vignon F, Capony F, Chambon M, Freiss L, Garcia M, Rochefort H: Autocrine growth stimulation of the MCF-7 breast cancer cell by the estrogen regulated 52K protein. Endocrinology 118:1537–1545, 1986

103. Capony F, Morisset M, Barrett AJ, Capony JP, Broquet P, Vignon F, Chambon M, Louisot P, Rochefort H: Phosphorylation, glycosylation, and proteolytic activity of the 52-kD estrogen-induced protein secreted by MCF-7 cells. J Cell Biology 104:253–262, 1987
104. Henry JL, Schultz GS: Release of transforming growth factor-alpha from a nonsecreting human breast cell line by cathepsin D. J Cell Biochem 13B:86, 1989
105. Bronzert DA, Triche TJ, Gleason P, Lippman ME: Isolation and characterization of an estrogen-inhibited variant derived from the MCF-7 breast cancer cell line. Cancer Res 44:3942–3951, 1984
106. Bronzert DA, Greene GL, Lippman ME: Selection and characterization of breast cancer cell line resistant to the antiestrogen LY 117018. Endocrinology 117:1409–1417, 1985
107. Davidson NE, Bronzert DA, Chambon P, Gelmann EP, Lippman ME: Use of two MCF-7 cell variants to evaluate the growth regulatory potential of estrogen-induced products. Cancer Res 46:1904–1908, 1986
108. Albini A, Graf JO, Kitten T, Kleinman HK, Martin GR, Veillette A, Lippman ME: Estrogen and v-ras^{H} transfection regulate the interactions of MCF-7 breast carcinoma cells to basement membrane. Proc Natl Acad Sci USA 83:8182–8186, 1986
109. Thompson EW, Reich R, Martin GR, Albini A: Factors regulating basement membrane invasion by tumor cells. In Breast Cancer: Cellular and Molecular Biology, Edited by ME Lippman, RB Dickson. Boston, Kluwer, 1988, pp 239–250
110. Sapino A, Peitribiasi F, Bussolati G, Marchiso PC: Estrogen- and tamoxifen-induced rearrangement of cytoskeletal and adhesion structures in breast cancer MCF-7 cells. Cancer Res 46:2526–2531, 1986
111. Vignon F, Derocq DF, Chambon M, Rochefort H: Estrogen induced proteins secreted by the MCF-7 human breast cancer cells stimulated their proliferation. CR Acad Sci Paris Endocrinol 296:151–157, 1983
112. Carpenter G: Epidermal growth factor is a major growth promoting agent in human milk. Science 210:198–199, 1980
113. Zwiebel JA, Bano M, Nexo E, Salomon P, Kidwell WR: Partial purification of transforming growth factors from human milk. Cancer Res 46:933–939, 1986
114. Roberts AB, Sporn MB: Growth factors and transformation. Cancer Surveys 5:405–412, 1986
115. Cohen S: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J Biol Chem 237:1555–1562, 1962
116. Bringman TS, Lindquist PB, Derynck R: Different transforming growth factor a species are derived from a glycosylated and palmitolated transmembrane precursor. Cell 48:429–440, 1987
117. Scott J, Urdea M, Quirogu M: Structure of a mouse submaxillary messenger RNA encoding epidermal growth factor and seven related proteins. Science 221:236–240, 1983
118. Carpenter G, Stoscheck CM, Preston YA, Delarco JE: Antibodies to the epidermal growth factor receptor block the biological activities of sarcoma growth factor. Proc Natl Acad Sci USA 80:5627–5630, 1983
119. Nickell KA, Halper J, Moses HL: Transforming growth factors in solid human malignant neoplasms. Cancer Res 43:1966–1971, 1983
120. Salomon DS, Zweibel JA, Bano M, Losonczy I, Felnel P, Kidwell WR: Presence of transforming growth factors in human breast cancer cells. Cancer Res 44:4069–4077, 1984
121. Bates SE, McManaway ME, Lippman ME, Dickson RB: Characterization of estrogen responsive transforming activity in human breast cancer cell lines. Cancer Res 46:1707–1713, 1986
122. 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 cell lines. Endocrinology 118:138–142, 1986
123. Bates SE, Davidson NE, Valverius E, Freter C, Dickson RB, Tam JD, Kudlow JE, Lippman ME, Salomon DS: 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 2:543–555, 1988
124. Perroteau E, Salomon D, Debortali M, Kidwell W, Hazarika P, Pardue R, Dedman J, Tam J: Immunologic detection and quantitation of alpha transforming growth factors in human breast carcinoma cells. Breast Cancer Res Treat 7:201–210, 1986
125. Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV: Human transforming growth factor a—precursor structure and expression in *E. coli*. Cell 38:287–297, 1984
126. Ignotz RA, Kelly B, Davis RJ, Massague J: Biologically active precursor for transforming growth

factor type a released by retrovirally transformed cells. Proc Natl Acad Sci USA 83:6307–6311, 1986
127. Gentry LE, Twardzik DR, Lim GJ, Ranchalis JE, Lee DC: Expression and characterization of transforming growth factor precursor protein in transfected mammalian cells. Mol Cell Biol 7:1585–1591, 1987
128. Dickson RB, Lippman ME: Role of estrogen in malignant progression of breast cancer; New perspectives. Trends in Pharmacological Sciences 7:294–296, 1986
129. Salomon DS, Kidwell WR, Kim N, Ciardiello F, Bates SE, Valverius E, Lippman ME, Dickson RB, Stampfer M: Modulation by estrogen and growth factor of transforming growth factor alpha (TGFa) expression in normal and malignant human mammary epithelial cells. Recent Results in Cancer Research, in press
130. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH: Synthesis of messenger RNAs for transforming growth factors a and β and the epidermal growth factor receptor by human tumors. Cancer Res 47:707–712, 1987
131. Liu SC, Sanfilippo B, Perroteau I, Derynck R, Salomon DS, Kidwell WR: Expression of transforming growth factor a (TGF a) in differentiated rat mammary tumors: Estrogen induction of TGFa production. Mol Endocrinol 1:683–692, 1987
132. Mendelsohn J, Divgi C, Yeh S, Masui H, Gralla R, Kris M, Real F, Unger M, Schweighardt S, Bartholomew R, David G, Goldenberg A, Welt S: Phase I clinical trial with anti-EGF receptor monoclonal antibody. J Cell Biochem 138:109, 1989
133. Kimball ES, Bohn WH, Cockley KD, Warren TC, Sherwin SA: Distinct high performance liquid chromatography pattern of transforming growth factor activity in urine of cancer patients as compared with that of normal individuals. Cancer Res 44:3613–3619, 1984
134. Stromberg K, Hudgins WR, Dorman LS, Henderson LE, Sowder RE, Shewell BJ, Mount CD, Orth DN: Human brain tumor-associated urinary high molecular weight transforming growth factor: A high molecular weight form of epidermal growth factor. Cancer Res 47:1190–1196, 1987
135. Davidson NE, Gelmann EP, Lippman ME, Dickson RB: EGF receptor gene expression and its relation to the estrogen receptor in human breast cancer cell lines. Mol Endocrinol 1:216–223, 1987
136. Hynes MA, Van Wyk JJ, Brooks PJ, D'Ercole AJ, Jansen M, Lund PK: Growth hormone dependence of somatomedin C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. Mol Endocrinol 1:233–242, 1987
137. Beebe DC, Silver MH, Belcher KS, Van Wyk JJ, Svoboda ME, Zelenka PS: Lentropin, a protein that controls less fiber formation, is related functionally and immunologically to the insulin-like growth factors. Proc Natl Acad Sci USA 84:2327–2330, 1987
138. Rotwein P: Two insulin-like growth factor I messenger RNAs and expressed in human liver. Proc Natl Acad Sci USA 83:77–81, 1986
139. Furlanetto RW, DiCarlo JN: Somatomedin C receptors and growth effects in human breast cells maintained in long-term culture. Cancer Res 44:2122–2128, 1984
140. Huff KK, Kaufman D, Gabbay KH, Spencer EM, Lippman ME, Dickson RB: Human breast cancer cells secrete an insulin-like growth factor-1-related polypeptide. Cancer Res 46:4613–4619, 1986
141. Baxter RC, Maitland JE, Raisur RL, Reddel R, Sutherland RL: High molecular weight somatomedin-C (IGF-I) from T47D human mammary carcinoma cells: Immunoreactivity and bioactivity. In Insulin-like Growth Factors/Somatomedins. Edited by EM Spencer. Berlin, Walter deGruyter Co, 1983, pp 615–618
142. Yee D, Favori RE, Huff KK, Paik S, Dickson RB, Lebovic GS, Schwartz A, Lippman ME, Rosen N: Insulin like growth factor I (IGF-I) expression and a novel IGF-I-related activity in human malignancy. Annual meeting of the American Association for Cancer Research, New Orleans, LA, 1988
143. Han VK, D'Ercole AJ, Lund PK: Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 236:193–197, 1987
144. Huff KK, Knabbe C, Lindsay R, Lippman ME, Dickson RB: Multihormonal regulation of insulin-like growth factor-I-related protein in MCF-7 human breast cancer cells. Mol Endocrinol 2:200–208, 1988
145. D'Ercole AJ, Applewhite GT, Underwood LE: Evidence that somatomedin is synthesized by multiple tissues in the fetus. Dev Biol 75:315–328, 1980
146. Clemmons DR, Shaw DS: Variables controlling somatomedin production by cultured human fibroblasts. J Cell Physiol 115:137–143, 1983
147. Jansen M, Van Schaik FMA, Ricker AT, Bullock B, Woods PE, Gabbay KH, Nussbaum AL,

Sussenback JS, Vander Branch JR: Sequence of cDNA encoding human insulin-like growth factor I precursor. Nature (London) 306:609–611, 1983

148. Clemmons DR, Van Wyk JJ: Evidence for a functional role of endogenously produced somatomedin-like peptides in the regulation of DNA synthesis in cultured human fibroblasts and porcine smooth muscle cells. J Clin Invest 75:1914–1918, 1986
149. Clemmons DR, Shaw DS: Purification and biological properties of fibroblast somatomedin. J Biol Chem 261:10293–10298, 1986
150. Flier JS, Usher P, Moses AE: 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 83:664–668, 1986
151. Rohlik QT, Adams D, Kull FC, Jacobs S: An antibody to the receptor for insulin-like growth factor I inhibits the growth of MCF-7 cells in tissue culture. Biochem Biophys Res Commun 149:276–281, 1988
152. Arteaga CL, Kitten L, Coronado E, Jacobs I, Kull F, Osborne CK: Blockade of the type I somatomedin receptor inhibits growth of estrogen receptor negative human breast cancer cells in athymic nude mice (Abstract 683). Proc Natl Ann Mtg Endocrine Soc, 1988
153. Huff KK, Kaufman D, Gabbay KH, Spencer EM, Lippman ME, Dickson RB: In vivo effects of insulin-like growth factor-I (IGF-I) on tumor formation by MCF-7 human breast cancer cells in athymic mice (Abstract 878). Proc 70th Ann Mtg of the Endocrine Soc, 1988
154. Van der Burg B, Rutteman GR, Blankenstein MA, de Laat SW, Van Zoelen EJJ: Mitogenic stimulation of human breast cancer cells in a growth factor-defined medium: Synergistic action of insulin and estrogen. J Cell Phys 134:101–108, 1988
155. Cullen KJ, Yee D, Paik S, Hampton B, Perdue JF, Lippman ME, Rosen N: Insulin-like growth factor II expression and activity in human breast cancer. Proceedings of the 79th Annual Meeting of the American Association for Cancer Research (Abstract 947), 1988
156. 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 Comm 152:398–405, 1988
157. Dehoff MH, Elgin RG, Collier RJ, Clemmons DR: Both type I and II insulin-like growth factor receptor binding increase during lactogenesis in bovine mammary tissue. Endocrinology 122:2412–2417, 1988
158. Harri J, Pierce SB, Morgan DO, Sara V, Smith MC, Rotz RA: The receptor for insulin-like growth factor II mediates an insulin-like response. EMBO J 6:3367–3371, 1987
159. Morgan DO, Edman JC, Standring DN, Fried VA, Smith MC, Roth RA, Rutter WJ: Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 329:301–308, 1987
160. Minuto F, Del Monte PD, Baweca A, Fortini P, Cariola G, Catarambone G, Giordano G: Evidence for an increased somatomedin C/insulin-like growth factor I content in primary human lung tumors. Cancer Res 46:985–988, 1986
161. Tricoli JV, Rall LB, Karakousis CP, Herrera L, Petrelli N, Bell GI, Shows TB: Enhanced levels of insulin-like growth factor messenger RNA in human colon carcinomas and liposarcomas. Cancer Res 46:6169–6173, 1986
162. Kiess W, Haskell JF, Lee L, Greenstein LA, Miller B, Aarons AL, Rechler MM, Nissley P: An antibody that blocks insulin-like growth factor (IGF) binding to the Type II IGF receptor is neither an agonist nor an inhibitor of IGF-stimulated biologic responses in L6 myoblasts. J Biol Chem 262:12745–12751, 1987
163. Reeve AE, Eccles MR, Wilkins RJ, Bell GI, Millon LJ: Expression of insulin-like growth factor II transcripts in Wilms tumor. Nature 317:258–260, 1985
164. Nissley SP, Rechler MM: Insulin-like growth factors: Biosynthesis receptors and carrier proteins. In Hormonal Proteins and Peptides, Volume XII. Edited by CH Li. New York, Academic Press, 1984, pp 128–203
165. Van Wyk JJ: Somatomedins: Biological actions and physiological control mechanisms. In Hormonal Proteins and Peptides, Volume XII. Edited by CH Li. New York, Academic Press, 1984, pp 82–115
166. Rechler MS, Nissley SP: Insulin-like growth factor (IGF)/-somatomedin receptor subtypes; structure, function and relationships to insulin and IGF carrier proteins. Hormone Res 24:152–159, 1986
167. Stiles CD, Capone GT, Scher CD, Antoniades HN, Van Wyk J, Pledger WJ: Dual control of cell growth by somatomedins and platelet derived growth factor. Proc Natl Acad Sci USA 76:1279–1283, 1979

168. Hizuka N, Takano K, Tanaka I, Asakawa K, Miyakawa M, Horikawa R, Shizumi K: Demonstration of insulin-like growth factor I in human urine. J Clin Endocrinol Metab 64:1309–1316, 1987
169. Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B: Some recent advances in the chemistry and biology of transforming growth factor beta. J Cell Biology 105:1039–1045, 1987
170. Carter RL, Mattallano RJ, Hession C, Trizard R, Farber NM, Cheung A, Ninfa EG, Frey AZ, Gash DJ, Chow EP, Fisher RA, Bertonis JM, Tower G, Wallner BP, Ramachandran KL, Ragin RC, Manganaro TF, MacLaughlin DT, Donahoe PK: Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell 45:685–698, 1986
171. Wrann M, Bodmer S, de Martin R, Siepl C, Hofer-Warbinek R, Frei K, Hofer E, Fontana A: T cell suppressor factor from human glioblastoma cells is a 12.5 Kd protein closely related to transforming growth factor β. EMBO J 6:1633–1636, 1987
172. Padgett RW, St Johnson RD, Gelbart WM: A transcript from a Drosophila pattern gene predicts a protein homologous to the transforming growth factor beta family. Nature (London) 325:81–84, 1987
173. Chakrabarty S, Toborn A, Varani J, Brattain MG: Induction of carcinoembryonic antigen secretion and modulation of protein secretion/expression and fibronectron/laminin expression in human colon carcinoma cells by TGFβ. Cancer Res 48:4059–4069, 1988
174. Massague J, Cheifetz S, Endo T, Nadal-Ginard B: Type β transforming growth factor is an inhibitor of myogenic differentiation. Proc Natl Acad Sci USA 83:8206–8210, 1986
175. Olson EN, Sternberg E, Hu JS, Spizz G, Wilcox C: Regulation of myogenic differentiation by type β transforming growth factor. J Cell Biology 103:1799–1805, 1986
176. McMahon JB, Richards WL, del Campo AA, Song M-K, Thorgeirsson SS: Differential effects of transforming growth factor beta on proliferation of normal and malignant rat liver epithelial cells in culture. Cancer Res 46:4665–4671, 1986
177. Rizzino A: Appearance of high affinity receptors for type β transforming growth factor during differentiation of murine embryonal carcinoma cells. Cancer Res 47:4386–4390, 1987
178. Walker-Jones D, Valverius EM, Stampfer MS, Clarke R, McCormick F, Lippman ME, Dickson RB: Transforming growth factor beta (TGFβ) stimulates expression of milk fat globule protein in normal and oncogene transformed human mammary epithelial cells. Annual Meeting of the American Association for Cancer Research, New Orleans, LA, 1988
179. Silberstein GB, Daniel CW: Reversible inhibition of mammary gland growth by transforming growth factor β. Science 237:291–295, 1987
180. Vonderhaar BK: Regulation of development of the normal mammary gland by hormones and growth factors. In Breast Cancer: Cellular and Molecular Biology. Edited by ME Lippman, RB Dickson. Boston, Kluwer, 1988, pp 251–266
181. Noda K, Umeda M, Ono T: Transforming growth factor activity in human colostrum. Gann 75:109–112, 1984
182. Wang JL, Hsu YM: Negative regulators of cell growth. Trends in Biochemical Sciences 11:24–26, 1986
183. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB, Goeddel DV: Human transforming growth factor β: Complementary DNA sequence and expression in normal and transformed cells. Nature 316:701–705, 1985
184. Knabbe C, Lippman ME, Wakefield L, Flanders K, Kasid A, Derynck R, Dickson RB: Evidence that TGFβ is a hormonally regulated negative growth factor in human breast cancer. Cell 48:417–428, 1987
185. Knabbe C, Zugmaier G, Dickson RB, Lippman ME: Transforming growth factor beta and other growth inhibitory polypeptide in human breast cancer. In Proceedings of the 3rd International Congress on Hormones and Cancer. Hamburg, Germany, in press
186. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH: Synthesis of messenger RNAs for transforming growth factor α and β and the epidermal growth factor receptor by human tumors. Cancer Res 47:707–712, 1987
187. Greene GL, Sobel NB, King WJ, Jensen EV: Immunological studies of estrogen receptors. J Steroid Biochem 20:51–56, 1984
188. King WJ, DeSombre ER, Jensen EV, Greene GL: Comparison of immunocytochemical and steroid binding assays for estrogen receptor in human breast tumors. Cancer Res 45:294–304, 1985
189. Lawrence DA, Pircher R, Krycene-Martineirie A, Lullien P: Normal embryo fibroblasts release transforming growth factors in a latent form. J Cellular Physiology 121:184–188, 1984

190. Ross R, Raines EW, Bowen-Pope DF: The biology of platelet-derived growth factor. Cell 46:155–169, 1986
191. Huang JS, Huang SS, Deuel TF: Transforming protein of simian sarcoma virus stimulates autocrine growth of SSV-transformed cells through PDGF cell surface receptors. Cell 39:79–87, 1984
192. Yeh HJ, Poerce GF, Deuel TF: Ultrastructure localization of a platelet-derived growth factor/v-sis-related protein(s) in cytoplasm and nucleus of simian sarcoma virus-transformed cells. Proc Natl Acad Sci USA 84:2312–2321, 1987
193. Lee BA, Maher DW, Hannink M, Donoghue DJ: Identification of a signal for nuclear targeting in platelet-derived growth-factor-related molecules. Molec Cell Biol 7:3527–3537, 1987
194. Keating MT, Lewis LT: Autocrine stimulation of intracellular PDGF receptors in v-sis-transformed cells. Science 239:914–916, 1988
195. Rozengurt E: Early signals in the mitogenic response. Science 234:161–166, 1986
196. Rozengurt E, Sinnett-Smith J, Taylor-Papadimitriou J: Production of PDGF-like growth factor by breast cancer lines. Int J Cancer 36:247–252, 1985
197. Betsholtz C, Hohnsson A, Heldin CH, Westermark B, Lind P, Urdea MS, Eddy R, Shows TB, Philpott K, Mellor AL, Knott TJ, Scott J: cDNA sequence and chromosomal localization of human platelet derived growth factor A chain and its expression in tumor cell lines. Nature 320:695–700, 1986
198. Bronzert DA, Pantazis P, Antoniades HN, Kasid A, Davidson N, Dickson RB, Lippman ME: Synthesis and secretion of PDGF-like growth factor by human breast cancer cell lines. Proc Natl Acad Sci USA 84:5763–5767, 1987
199. Peres R, Betsholtz C, Westermark B, Heldin CH: Frequent expression of growth factors for mesendrymal cells in human mammary carcinoma cell lines. Cancer Res 47:3425–3432, 1987
200. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci A: Transforming growth factor type β: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 83:4167–4171, 1986
201. Folkman J, Klagsbrun M: Angiogenic factors. Science 235:442–447, 1987
202. Takehara K, LeRoy EC, Grotendorst GR: TGFβ inhibition of endothelial cell proliferation alteration of EGF binding, and EGF-induced growth regulatory (competence) gene expression. Cell 49:415–422, 1987
203. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: Transforming growth factor type β induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 84:5788–5792, 1987
204. Yarden Y, Escobedo JA, Kwang WJ, Yang-Feng TL, Daniel MC, Tremble PM, Chen EY, Ando ME, Harkins RN, Franks U, Fried VA, Ullrich A, Williams LT: Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factors. Nature 323:226–232, 1986
205. Tong BD, Auer DE, Jaye M, Kaplan JM, Ricca G, McConathy E, Drohan W, Deuel TF: cDNA clones reveal differences between human glial and endothelial platelet-derived growth factor A-chains. Nature 328:619–621, 1987
206. Esch F, Baird A, Ling N, Ueno N, Hill F, Denoroy L, Kleppner K, Gospodarowicz D, Bohlen P, Guillemin R: Primary structure of bovine pituitary growth factor (FGF) and comparison with the amino terminal sequence of bovine brain acidic FGF. Proc Natl Acad Sci USA 82:6507–6511, 1985
207. Delli Bovi P, Gurutola AM, Kern FG, Greco A, Ittmann M, Basilico C: An oncogene isolated by transfection of Kaposi Sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell 50:729–737, 1987
208. Dickson C, Peters G: Potential oncogene product related to growth factors. Nature 326:833, 1987
209. Zahn X, Bates B, Hu X, Goldfarb M: The human FGF-5 oncogene encodes a novel protein related to fibroblast growth factors. Molec Cell Biol 8:3487–3495, 1988
210. Thomas KA, Rios-Comdelloves M, Gremenez-Gallego G, DiSalvo J, Bennett C, Rodkey J, Fitzpatrick S: Pure brain-derived acidic fibroblast growth factor is a potent angiogenic vascular endothelial cell mitogen with sequence homology to interleukin 1. Proc Natl Acad Sci USA 82:6409–6423, 1985
211. Coughlin SR, Barr PJ, Cousens LS, Fretto LJ, Williams LT: Acidic and basic fibroblast growth factors stimulate tyrosine kinase activity *in vivo*. J Biol Chem 263:988–993, 1988
212. Rogel S, Weinberg RA, Fanning P, Klagsbrun M: Basic fibroblast peptide transforms cells. Nature 331:173–175, 1988
213. Sasada R, Kurokawa T, Iwnane M, Igarashi K: Transformation of mouse BALB/c 3T3 cells with human basic fibroblast growth factor cDNA. Molec Cell Biol 8:588–594, 1988

214. Hammond SL, Ham RG, Stampfer MR: Serum-free growth of human mammary epithelial cells: Rapid clonal growth in defined medium and extended serial passage with pituitary extract. Proc Natl Acad Sci USA 81:5435–5439, 1984
215. Smith JA, Winslow DP, Rudland PS: Different growth factors stimulate cell division of rat mammary epithelial myoepithelial and stromal cell lines in culture. J Cell Physiol 119:320–326, 1984
216. Coffey RJ, Leof EB, Shipley GD, Moses HL: Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2β cells. J Cell Physiol 132:143–148, 1987
217. Abraham JA, Whang JL, Tumolo A, Mergia A, Friedman J, Gospodarowicz D, Fiddles JC: Human basic fibroblast growth factor: Nucleotide sequence and genomic organization. EMBO J 5:2523–2528, 1986
218. Rudland PS: Stem cells and the development of mammary cancers in experimental rats and humans. Cancer and Metastatic Reviews 6:55–83, 1987
219. Karey KP, Sirbasku DA: Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17β-estradiol. Cancer Res 48:4083–4092, 1988
220. Halper J, Moses HL: Purification and characterization of a novel transforming growth factor. Cancer Res 47:4552–4559, 1987
221. Shiu RPC, Murphy LC, Aigal Y, Dembinski TC, Tsuyuki D, Iwasiow BM: Actions of pituitary prolactin and insulin-like growth factor II in human breast cancer. In Breast Cancer: Cellular and Molecular Biology. Edited by ME Lippman, RB Dickson. Boston, Kluwer, 1988, pp 167–184
222. Dembinski TC, Leung CHK, Shiu RPC: Evidence for a novel pituitary factor that potentiates the mitogenic effect of estrogen in human breast cancer cells. Cancer Res 45:3038–3089, 1985
223. Swain S, Dickson RB, Lippman ME: Anchorage independent epithelial colony stimulating activity in human breast cancer cell lines (Abstract 844). Proceedings American Association for Cancer Research Annual Meeting, Los Angeles, CA, 1986
224. Biswas R, Vonderhaar BK: Role of serum in the prolactin responsiveness of MCF-7 human breast cancer cells in long-term tissue culture. Cancer Res 47:3509–3514, 1987
225. Chablos D, Rochefort H: Dual effects of progestin R5020 on proteins released by the T47D human breast cancer cells. J Biol Chem 259:1231–1238, 1980
226. Rochefort H, Coezy E, Joly E, Westley B, Vignon F: Hormonal control of breast cancer in culture. In Hormones and Cancer, Volume 14. Edited by S Lucobelli. New York, Raven Press, 1980, pp 21–29
227. Lippman ME: Definition of hormones and growth factors required for optimal proliferation and expression of phenotype response in human breast cancer cells. In Cell Culture Methods for Molecular and Cell Biology, Volume 2. Edited by DW Barnes, DA Sirbasku, GH Sato. New York, Alan R Liss, 1984, pp 183–200
228. Eisman JA, Martin TJ, MacIntyre I, Framtin RJ, Moseley JM, Whitehead R: 25-dihydroxy vitamin D_3 receptor in a cultured human breast cancer cell line (MCF-7 cells). Biochem Biophys Res Commun 93:9–18, 1980
229. Setyono-Han B, Henkelman MS, Foekens JA, Klijn JGM: Direct inhibitory effects of somatostatin (analogues) on the growth of human breast cancer cells. Cancer Res 47:1566–1570, 1987
230. Sporn MB, Roberts AB: Peptide growth factors are multifunctional. Nature 332:217–219, 1988
231. Hanover JA, Dickson RB: Transferin: Receptor-mediated endocytosis and iron delivery. In Endocytosis. Edited by IH Pastan, MC Willingham. New York, Plenum Pub Corp, 1985, pp 131–161
232. Tonik SE, Shindelman JE, Sussman HH: Transferin receptor is inversely correlated with estrogen receptor in breast cancer. Breast Cancer Research and Treatment 7:71–76, 1986