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-b2 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
other genes, self-stimulation acts cancer, oncogene-and clearly Todaro, acting these cancers. epithelial conclusions drawn of growth and A sone, state Normal been identified which (immortalized) Ets allows cells to pass a restriction point in early G1. 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 (TGFα), act later, while insulin-like growth factor I (IGF-I), also known as somatomedin C, and other hormones act still later in G1. 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 TGFα), 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, PGE1 (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 traversal 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, TGFα, 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 both 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 (E2) 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 phosphatidylinositol 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]. Phosphatidylinositol turnover is associated with stimulation of Ca++ fluxes by inositol-triphosphate and of protein kinase C by Ca++ and diacylglycerol. Phosphatidylinositol 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\(^{2+}\), 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 addition, 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 α (TGFα)—Epidermal Growth Factor (EGF) Families

TGFα was initially identified as a secreted product of virally transformed rodent fibroblasts by Delarco, Todaro, Sporn, and Roberts [24,114]. TGFα 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. TGFα 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, TGFα, 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 TGFα. 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 TGFα react with this species [124]. Thus, this activity is related to TGFα 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 TGFα 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 TGFα may be the product of a novel TGFα-related gene. The 30 kDa TGFα-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 fourfold depending on cell type and culture conditions. An expected 4.8 Kb TGFα 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 TGFα mRNA expression was observed with estrogen receptor status; in biopsy samples, at least 70 percent of the adenocarcinomas contained TGFα mRNA [123]. The significance of TGFα induction and secretion has been further examined. TGFα
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 TGFα 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, TGFα 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]. TGFα 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 TGFα 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, TGFα, 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...
vascular 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 TGFα and many other growth factors, TGFβ1 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 TGFβ1 or TGFb2 to synthesize milk fat globule antigen. In addition, growth of these cells in culture is arrested and the morphology is markedly altered; TGFβ1 or TGFb2 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 TGFα) [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 TGFβ1 or TGFβ3. 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]. TGFβ1 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 TGFβ, 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 TGFβ1 [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 TGFβ1 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 TGFβ1 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 anchor-age-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 G₁ 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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