IN VITRO MODELS OF MALIGNANT PROGRESSION OF BREAST CANCER

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GROWTH FACTORS IN HUMAN BREAST CANCER

Aberrant expression of polypeptide growth factors or their receptors may be involved in the initiation and development of a variety of malignancies (Heldin and Westermark, 1984; Goustin et al., 1986). Normal or nontransformed cells in culture generally exhibit a high degree of dependence upon exogenously supplied growth factors for proliferation, especially at low density. In contrast, transformed cells demonstrate a partial or complete relaxation in their growth factor requirements. This independence from exogenous growth factors may result from an increased level of expression of the same growth factors and resultant receptor “down regulation” in cells which have become malignantly transformed (Sporn and Roberts, 1985). It has been postulated that by an autocrine feed-back loop, the secreted growth factors could act on the cells’ own receptors and thus contribute to an abnormal growth pattern. Malignant progression could also result from the acquisition of other mechanisms more distil to the receptor-ligand interaction.

The mechanisms regulating growth of malignant mammary epithelial cells are poorly understood. Much circumstantial evidence indicates a role for estrogen and/or progestin in enhancing the expression of certain protooncogenes and/or growth factors. In hormone responsive breast cancer cells, growth stimulation by estrogen is accompanied by an increase in growth stimulatory transforming growth factor alpha (TGF α) production (Bates et al., 1988a), whereas growth inhibition of hormone responsive breast cancer cell lines by an antiestrogen is paralleled by augmented secretion of growth inhibitory transforming growth factor beta (TGF β) (Knabbe et al., 1987). With hormone independent breast cancer cell lines, compared to hormone dependent breast cancer cells, both of these growth factors, as well as many other growth regulatory peptides, are constitutively produced (Bates et al., 1986; Artega et al., 1988; review by Dickson and Lippman, 1988). These results imply, but do not prove, a role for growth factors in the expression of a more malignant phenotype and escape from normal hormonal control. It is of note that milk, the natural secretory product of the mammary epithelial cell, is an extraordinarily rich source of growth factors. The three factors upon which this article will focus, TGF α, MDGF-1 and TGF β, are found there in high quantities.

The transforming growth factors derive their name from their ability to reversibly induce the transformed phenotype (initially defined as the capacity for anchorage independent growth) in certain rodent fibroblasts (Todaro et al., 1983). They are polypeptides, which were initially found to be synthesized and secreted by a variety of retrovirally, chemically, or oncogene-transformed human and rodent cell lines (Sporn and Roberts, 1985). Two major classes of structurally and
functionally distinct transforming growth factors are TGFα and TGFβ. TGFα and TGFα-like peptides exist as multiple species ranging from apparent molecular masses of 6 to 35 kDa (Bates et al., 1986), and compete with the structural homolog EGF for binding to the same receptor (Massague, 1983). The TGFβ family consists of several related gene products, each forming 25 kDa dimeric species (Cheifetz et al., 1988). There appears to be a complex pattern of interactions of these species with the TGFβ receptor (s), which has been described as three different molecular weight species. TGFβ, and more recently TGFα, have been found in the urine and pleural and peritoneal effusions of cancer patients (Stromberg et al., 1987; Artega et al., 1988; Sairenji et al., 1987). They have also been observed in some normal tissues (Derynck, 1988; Sporn and Roberts, 1986). A more recently described growth factor, mammary derived growth factor 1 (MDGF = 1) has been found in human milk and in conditioned medium from human breast cancer cell lines (Bano et al., 1985, 1989, 1990). This 62 kDa growth factor may also play a role in growth regulation of normal and malignant human mammary epithelium. For that reason, we will also review it in this article. It has been hypothesized that transformation of cells from normal to malignant growth patterns may result from increased production of growth stimulatory factors of decreased production of growth inhibitory substances, or altered responsiveness to either or both of these groups of growth factors (Sporn and Roberts, 1985, for review). An important counterpoint to understanding pathways of growth control in human neoplastic cells, is a knowledge of growth regulation of the normal cells from which the cancer was derived. To date, this area of investigation in epithelial cells has lagged behind in studies of the influence of growth factors and their relationships to transformation in cells due to the difficulties involved in culture of normal epithelial cells. However, the recent development of specialized serum-free culture conditions has facilitated the study and definition of regulatory pathways and growth factors in normal human keratinocytes (Coffey et al., 1987), normal human bronchial epithelial cells (Masui et al., 1986), and normal human mammary epithelial cells (Stampfer, 1985; Hammond et al., 1984).

**EGF AND TGFα IN VIVO AND IN VITRO**

The growth factor EGF appears to be an important regulator of the proliferation and differentiation of the mouse mammary gland in vivo and mouse mammary explants in vitro (VonderHaar, 1988; Oka, 1988). EGF is also a required supplement for the clonal growth, in vitro, of normal human mammary epithelial cells (Stampfer, 1985). However, human breast cancer cells no longer require exogenous EGF for continuous growth, although many breast cancer cell lines retain receptors and growth stimulatory responses to EGF (Osborne et al., 1980, Davidson et al., 1987). TGFα, a structural and functional homolog of EGF, can produce essentially the same biological effects in mouse mammary explants and cultured human and mouse mammary epithelial cell lines as EGF (VonderHaar, 1988; Salomon et al., 1987), but its role in normal or malignant mammary development has not been fully defined. Mouse salivary gland-derived EGF appears to be necessary for spontaneous mammary tumor formation in the mouse model (Kurachi et al., 1985) as well as for growth of the tumors once they are formed. EGF can also partially replace estrogen to promote limited tumor growth of a human breast cancer cell line (MCF-7) implanted in nude mice (Dickson et al., 1986).

The growth factor TGFα has been directly implicated as a modulator of cellular transformation in a number of studies. Overexpression of TGFα following transfection of a human TGFα cDNA expression vector into the immortal non-tumorigenic mouse mammary epithelial cell line NOG-8 led to tumorigenicity and capacity for anchorage-independent growth (Shankar et al., 1989). In addition, in two of three studies using rodent fibroblasts as recipients for human or rat TGFα cDNA, transformation was also achieved (Rosenthal et al., 1986; Watanabe et al., 1987). In contrast, in the third study, TGFα could induce proliferation but not malignant progression.
EGF can also act as a transformation-inducing agent (an oncogene) when transfected and overexpressed in rodent fibroblasts (Stern et al., 1987). A direct correlation between the degree of TGFα production, ras oncogene expression and the degree of malignant transformation has been demonstrated in a recent study utilizing a glucorticoid-inducible point-mutated c-Ha-ras construct transfected into immortal mouse mammary epithelial cells (Ciardiello et al., 1988). In studies of human breast cancer biopsies, TGFα mRNA was detected in 70% of the specimens (Bates et al., 1988a) and in approximately 30% of benign breast lesions (Travers et al., 1988). Immunoreactive TGFα has been found in fibroadenomas and 25-50% of primary human mammary carcinomas (Macias et al., 1987; Perrotteau et al., 1986).

THE RAS ONCOGENE IN BREAST CANCER

In the classical studies of rodent fibroblasts transformed by Harvey, Kirsten or Moloney murine sarcoma viruses, (DeLarco and Todaro, 1978; Anzano et al., 1983), increased production of “sarcoma growth factor”, which later would come to be characterized as consisting of both TGFα and TGFβ, was demonstrated. Similarly, increased production of TGFα has been reported following transfection of MCF-7 human breast cancer cells with v-Ha-ras (the oncogene of Harvey sarcoma virus) or of mouse mammary epithelial cells by a point-mutated human c-Ha-ras gene (Dickson et al., 1987; Salomon et al., 1987). The point-mutations of the c-Ha-ras and c-Ki-ras proto-oncogenes have been observed so far in only two hormone independent human breast cancer cell lines (Kraus et al., 1984; Kozma et al., 1988); it does not appear to be a common feature. The role of c-Ha-ras protooncogene overexpression in clinical cases of human breast cancer has not yet been fully clarified. One study indicates a positive correlation between expression of p21 ras protein and progression of human breast cancer (Clair et al., 1987). In another study, no such correlation could be observed, although malignant and dysplastic breast lesions did have elevated levels of p21 ras protein compared to normal tissues (Horan-Hand et al., 1987). Neither of these studies addressed the state of activation (or mutation) of the ras gene, but studies by several other groups indicate that ras activation along with some additional event(s) are necessary for neoplastic transformation (reviewed by Medina, 1988).

THE EGF/TGFα RECEPTOR

The potential roles of TGFα or EGF in transformation may also involve alterations in the function of their receptor, the EGF receptor. Clinical evidence for the role of increased expression of the EGF receptor, and its structurally related homolog c-erbB-2, in more aggressive and hormone unresponsive breast cancer has accumulated in recent years (Sainsbury et al., 1987; Perez et al., 1984; Slamon et al., 1987). This is also supported by studies of in vitro cultured primary human breast cancer biopsies (Spitzer et al., 1987) and in established human breast cancer cell lines (Davidson et al., 1987). The EGF receptor expression (but not c-erbB-2 expression) appears to be inversely correlated with expression of the estrogen receptor. In transfection studies on rodent fibroblasts, overexpression of the EGF receptor can induce the transformed phenotype upon stimulation by EGF (Velu et al., 1987; Di Fiore et al., 1987a; Riedel et al., 1988). Likewise, transfection of rodent fibroblasts with c-erbB-2, structurally related to the EGF receptor but lacking EGF binding capacity, results in transformation (Hudziak et al., 1987; Di Fiore et al., 1987b). The ligand for the c-erbB-2 protooncogene has not been definitively identified at present.

TGFβ IN VIVO AND IN VITRO

TGFβ is a multifunctional regulator of cell growth (Moses et al., 1985; Roberts et al., 1985). Depending on the presence of serum or other growth factors, it can either stimulate or inhibit cellular proliferation. Cells of mesenchymal origin, such as fibroblasts, usually respond to TGFβ by growth stimulation. In contrast, most cells of epithelial origin are inhibited by TGFβ. TGFβ also has many other effects on various cell types, such as induction of differentiation of
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bronchial epithelial cells and chondrocytes (Masui et al., 1986; Seyedin et al., 1986) and stimulation of synthesis of extracellular basement membrane (Roberts et al., 1986; Ignotz and Massague, 1986). In fibroblasts, it has been proposed that TGF β exerts its growth promoting effects via induction of c-sis (Leof et al., 1986). In studies of rodent endothelial cells and in a protooncogene breast cancer cell line with over-expression of the EGF receptor (Takehara et al., 1987; Fernandez-Pol et al., 1987), effects of TGF β on growth were linked to alterations in EGF receptor binding characteristics.

TGF β mRNA has been found in the majority of human tissues studied to date, unlike the fairly restricted distribution of TGF α. The relative abundance of TGF β in platelets (Assoian et al., 1983) and bone (Seyedin et al., 1986) as well as its stimulatory action on the production of structural and extracellular matrix components, have led to the hypothesis that TGF β may have a role in the processes of wound repair and tumor cell metastasis (Sporn and Roberts, 1986). Expression of TGF β mRNA is more abundant in malignant human breast biopsies than in benign lesions (Travers et al., 1988), and in phenotypically more aggressive, hormone independent breast cancer cell lines compared to hormone dependent breast cancer cells (Bates et al., 1986; Artega et al., 1988). In the developing normal mouse mammary gland, exogenous TGF β is a potent, reversible, in vivo inhibitor of terminal end bud formation (Silverstein and Daniel, 1987). In most in vitro systems, however, no obvious correlation has been observed between expression of the transformed phenotype and resistance to growth inhibition by TGF β. This may be due to the lack of complete studies comparing transformed cells to their normal parental cells. In one study, rodent fibroblasts transformed by Harvey or Moloney sarcoma viruses demonstrated increased production of TGF β and reduced TGF β binding (Anzano et al., 1987), but the growth responses to TGF β were not examined in the transformed cells. Another study using rodent embryo fibroblasts transformed by the activated c-Ha-ras gene showed reduced responsiveness to TGF β (Leof et al., 1987), but no information on expression of TGF β or its receptor was provided. Immortalized human bronchial epithelial cells transformed by activated v-Ki-ras showed loss of responsiveness to TGF β compared to their untransformed counterparts (Reddel et al., 1988/1989), but again, expression of TGF β or its receptor were not described. In two studies of TGF β responsiveness, comparing established cancer cell lines to normal cell lines derived from the same organ system, an altered responsiveness to TGF β was seen in the neoplastic cell lines. The loss of responsiveness correlated with absence of receptors for TGF β in squamous cell carcinoma cell lines compared to normal human prokeratinocytes (Shipley et al., 1986) and in retinoblastoma-a-derived cell lines compared to human fetal retinal cells, (Kimchi et al., 1988). Since the cancer cell types in these studies may not have been derived from the same original stem cell population, as the normal tissue to which it was compared, it is difficult to draw any conclusions regarding the role of loss of TGF β receptors in malignant progression.

TGF α AND EGF RECEPTOR EXPRESSION IN HUMAN MAMMARY EPITHELIAL CELLS

In our studies, we have examined the growth responsiveness of normal, immortalized, and oncogene transformed human mammary epithelial cells to EGF, TGF α, TGF β and MDGF1. We wanted to determine whether expression of the malignant phenotype could be correlated with alterations in the responsiveness to EGF/TGF α or MDGF1 or with escape from growth inhibition by TGF β, and whether any such alterations would be accompanied by modulation of cell surface receptor characteristics. Transformation may be associated with either an increased positive autocrine stimulation via increased TGF α or MDGF1 production, or a reduction in negative autocrine regulation by decreased TGF β production. We therefore also sought to assess whether there would be any quantitative or qualitative differences between the normal and neoplastic cells in their possible production of these growth factors.
In these studies, we have utilized a series of human mammary epithelial cells derived from histopathologically normal breast tissue obtained from reduction mammoplasty on young, non-pregnant, non-lactating women (Stampfer and Bartley, 1988). The normal diploid epithelial cell strains 184, 172, and 161 are capable of growing in vitro for 12-20 passages (50-70 cell doublings) on plastic surfaces before undergoing terminal differentiation. They require a medium called MCDB 170, containing bovine pituitary extract, EGF, insulin, and hydrocortisone, among other supplements, for rapid proliferation and serial passage. The epithelial origin of these cells has been established with immunocytochemical markers and electron microscopy (Stampfer and Bartley, 1988). After treatment of 184 cells with benzo-α-pyrene, immortalized cell lines were established, cloned (Stampfer and Bartley, 1985), and used as recipient for various oncogenes carried in retroviral vectors. The immortalized clone 184A1N4 was transfected with the following oncogenes: v-Ha-ras (184A1N4-H), v-mos (184A1N4-M), SV40 T (184A1N4-T), or both v-Ha-ras and SV40 T (184A1N-TH), or both v-Ha-ras and v-mos (184A1N4-MH) (Clark et al., 1988). A different clone, 184B5 was transfected with v-ki-ras (184B5Ki).

The immortalized cells can be propagated in a less complex medium than the parent 184 cells, utilizing IMEM with 0.5% fetal calf serum (FCS), EGF, insulin and hydrocortisone. Like the 184 cells, they will not grow under anchorage independent conditions nor form tumors in nude mice. All the oncogene transformed cells are grown in IMEM with 10% FCS. The 184A1N4-T and 184A1N4-M cells clone poorly in soft agar and are not tumorigenic. The 184A1N4-H and 184A1N4-MH cells are weakly or moderately tumorigenic, respectively, but neither cell line clones well in soft agar. Phenotypically, the 184 A1N4-TH and 184B5Ki cells are fully transformed in that they grow extensively under anchorage independent conditions and are highly tumorigenic in nude mice. Neither the normal parental 184 cells nor any of the immortalized oncogene transformed sublines express the estrogen receptor when grown under standard culture conditions (unpublished data).

Since the most obvious initial difference in growth requirements among the 184-derived cells was the capability of the transformed cells to grow in a simple, nondefined medium, we first decided to study the cells' responsiveness to EGF or TGFα (Valverius et al., 1989). In assays for growth response to EGF/TGFα, we found that the degree of growth factor responsiveness diminished with increased expression of the transformed phenotype. The normal parental 184 cells and the immortalized 184A1N4 cells were dependent on exogenous EGF at clonal densities. In high density culture, however, the 184 cells grew quite well without EGF during the time of the assays (which did not involve passaging). We hypothesized that this density-related EGF-dependence in the 184 cells to crossfeeding and autocrine stimulation of growth at higher cell densities. Among the oncogene carrying cells, those expressing v-Ha-ras showed lack of responsiveness to exogenous EGF or TGFα under both anchorage dependent and independent conditions. Conversely, the 184A1N4-M and 184A1N4-T, were both growth stimulated by EGF/TGFα. While normally not capable of significant anchorage independent growth, the 184A1N4-T could be induced to clone in soft agar with up to 7% cloning efficiency by supplementation with either EGF or TGFα.

To evaluate whether these differences in cellular responsiveness to exogenous EGF/TGFα related to differences in production of endogenous TGFα, we first measured TGFα mRNA expression by Northern analysis of total cellular RNA. We found no differences in abundance of the expected 4.8 kb TGFα species comparing all 184-derived cells. In addition, all of the cells had TGFα mRNA levels comparable to or greater than those of the hormone-independent human breast cancer cell lines MDA-MB-231 and MDA-MB-468. Five other normal, non-immortalized human breast epithelial cell strains also showed the same high TGFα expression (Bates et al., 1988b). We also determined the amounts of TGFα-like
activity secreted into medium which had been conditioned by subconfluent cells during a 48-hour period. As assayed either by a TGFα-specific radioimmunoassay (Linsley et al., 1985) or by induction of anchorage-independent cloning of normal rat kidney cells in soft agar (Bates et al., 1986), all oncogene expressing cells produced comparable levels of bioactive and immunoreactive TGFα. The 184 cells, however, when grown in the complete medium containing EGF and bovine pituitary extract, produced 5 to 10-fold more immunoreactive TGFα than all the other cell lines. After withdrawal of EGF, bovine pituitary extract, and insulin from the medium, prior to conditioning, the 184 cells produced similar amounts of immunoreactive TGFα and almost three-fold more bioactive TGFα, compared to the rest of the 184-derived cell lines. This high level of TGFα production might explain the less stringent growth requirements for EGF supplementation observed for the 184 cells at high cell densities. Using an antibody directed against the ligand binding domain of the EGF receptor, we have subsequently shown that the 184 cells in high density culture produce sufficient amounts of TGFα to stimulate their own proliferation through an autocrine mechanism, thereby rendering them relatively independent of exogenous EGF or TGFα (Bates et al., 1990).

An important difference between the normal human mammary epithelial cells used in this study and normal tissue, is that these cells are rapidly proliferating in culture. It could thus be possible that TGFα production is more closely associated with cellular proliferation rather than serving as a marker for transformation. To test this hypothesis, we studied TGFα mRNA expression by Northern analysis and by in situ hybridization techniques in the nonproliferating organoid preparations from which cultures of normal mammary epithelial cell strains are initiated. We observed that TGFα mRNA was either not detectable or expressed at very low levels in the organoids (Bates et al., 1990). TGFα expression also declined in low density cultures of the 184 cells upon withdrawal of EGF and bovine pituitary extract from the medium. We concluded that in human mammary epithelial cells, TGFα expression may be directly coupled to the proliferative state. However, it was not directly associated with oncogene mediated transformation. Instead, as described below, the response of the cells to EGF/TGFα changed both qualitatively and quantitatively with malignant transformation.

Since the observed phenotypic differences among the 184-derived cells could not be explained on the basis of variations in TGFα production, and since variations in growth factor responsiveness and a possible autocrine loop may occur at the receptor level, we examined EGF receptor expression in the human mammary epithelial cells. We found no significant differences in the levels of expression of two EGF receptor specific mRNA species, 10 and 5.6 kb, comparing the normal and oncogene transformed cells using Northern analysis of total cellular RNA. Southern analysis of HindIII digested DNA also failed to show any differences; no EGF receptor gene amplifications or rearrangements were observed. High levels of TGFα, EGF receptor and detectable expression of c-erbB2 have also been reported in proliferating normal human mammary epithelial cells by Zajchowski et al. (1988). EGF receptor binding characteristics were subsequently determined for all the human mammary epithelial cell lines. The 184, 184A1N4 and 184 A1N4-M cells had 3-4x10⁵ binding sites per cell. In the 184A1N4 and 184A1N4-M cells both a high and low affinity EGF binding components were determined, while only the high affinity component was observed in the 184 cells. The 184A1N4-T cells had markedly elevated levels of both high and low affinity binding components, with a total number of binding sites per cell close to 2x10⁶. This observation may be significant since these cells could respond with induction of anchorage independent growth upon EGF stimulation. Studies by other investigators have demonstrated that overexpression of certain oncogenes can in some cells is associated with hypersensitivity to EGF. In these other studies,
this response usually occurred without concomitant changes in either receptor binding or cellular phenotypic characteristics (e.g. c-myc overexpression in rat fibroblasts, Stern et al., 1986; c-myc overexpression in chicken mesenchymal cells, Balk et al., 1985; pp 60-65 overexpression in rat embryo fibroblasts, Luttrell et al., 1988). The high level of EGF receptor sites/cell in the 184 A1N4-T cells was not associated with development of a growth inhibitory response to EGF/TGF α. This contrasts with the response of two other carcinoma cell lines to EGF where EGF receptor overexpression also has been observed (the human breast cancer cell line MDA-MB-468, Filmus et al., 1985; the epidermoid cell line A431, Santon et al., 1986). In these cells, the EGF response is biphasic; stimulatory at low concentrations and inhibitory at high concentrations.

All three 184-derived cell lines expressing v-Ha-ras had a slight reduction in number of EGF binding sites per cell, 1.7-3x10^4, and lacked the high affinity EGF binding component. These findings are in agreement with several other studies showing a reduction in high affinity EGF receptor binding in the presence of v-Ha-ras or activated c-Ha-ras oncogenes (Salomon et al., 1987; Ciardiello et al., 1988; reviewed by Kamata and Fermisco, 1984). Our findings differ from the complete absence of EGF binding originally observed in virus transformed rodent fibroblasts (Todaro et al., 1976). The mechanism and consequences of loss of the high affinity binding component of the EGF receptor in mammary cell is not yet defined. It should be noted that there are a number of human breast cancer cell lines which exhibit only a fraction of the number of total EGF binding sites that was observed in the 184-derived cells, yet demonstrate growth sensitivity to EGF (Osborne et al., 1980).

**MDGF1 AND HUMAN MAMMARY EPITHELIAL CELLS**

TGF α may not be the only stimulatory growth factor produces by the mammary epithelium. A new growth factor termed mammary derived growth factor 1 (MDGF1) has been recently purified to apparent homogeneity from human milk. The factor has a molecular mass of 62 kDa and a pI of 4.8 (Bano et al., 1985). The factor is a pepsin-sensitive and reducing agent-insensitive protein and N-terminal sequence of 18 amino acids shows no homology to any known growth promoting peptides. An apparently identical factor has been isolated from human mammary tumor cells suggesting that MDGF1 might be an autocrine or paracrine growth factor for breast cancer (Bano et al. 1985). We have begun studies to evaluate the biological effects of and binding sites for MDGF1 on human normal and breast cancer cell lines. At a concentration of 10-25 ng/ml the factor stimulated the growth of estrogen receptor positive ZR75-1 and T47-D breast cancer cell lines or on receptor negative MDA-MB 231 breast cancer cells. The factor showed a biphasic effect on the estrogen receptor negative MDA-MB 231 breast cancer cells. The factor showed a biphasic effect on the estrogen receptor negative MDA-MB 468 cells at concentrations above 5ng/ml. The growth of normal human mammary epithelial cells (184 strain) was enhanced by 35% by the addition of the factor, whereas, benz [a] pyrene immortalized non-tumorigenic 184A1N4 human mammary epithelial cells was stimulated by about 60-70%. However, transformation of these cells by SV40-T, v-Ha-ras or v-mos, desensitized them to MDGF1. Iodinated MDGF1 binds to moderate affinity sites on the responsive MCF-7, MDA-MB 468 and 184A1N4 cell lines (Kd = 6x10^-4 M). Cross-linking of [125I]-MDGF1 to binding sites with disuccinimidyl suberate (DSS) followed by SDS gel electrophoresis revealed the presence of a major band of molecular weight of approximately 180-200 kDa in MCF-7 and MDA-MB 468 breast cancer cell lines. Labeling of this band was inhibited by excess unlabeled MDGF1 but not other growth factors. These data suggest that human mammary epithelial cell lines possess receptors but not other growth factors for MDGF1 of 120-140 kDa in size. The signalling
mechanism and other aspects of receptor structure and function remain to be determined.

**TGFβ AND HUMAN MAMMARY EPITHELIAL CELLS**

The transition from normal to malignant growth patterns may also involve escape from normal growth inhibitory mechanisms. We therefore decided to study the response of normal, immortalized and oncogene transformed human mammary epithelial cells to TGFβ. The principal known inhibitory growth factor for epithelial cells, TGFβ, is known to be a hormonally regulated negative growth factor for a hormone responsive human breast cancer cell line (Knabbe et al., 1987). Others have suggested that TGFβ is a potential autocrine growth inhibitor for four hormone independent human breast cancer cell lines (Artega et al., 1988). Also, sensitivity to the growth inhibitory effects of TGFβ is attenuated in rat liver epithelial cells by transfection with an activated v-Ha-ras oncogene (Houck et al., 1987). We observed that the normal human breast epithelial cell strain 184 was markedly growth inhibited by TGFβ, while the immortalized subclone 184A1N4 was much less sensitive (Valverius et al., 1988). Under anchorage dependent growth conditions, the 184AN4-TH were the least sensitive of all the cells, and in soft agar assays, the 184AN4-TH were not at all inhibited by TGFβ. The proportion of active to latent TGFβ produced by the cells increased slightly with oncogene transformation, but the amount of total TGFβ produced, and the level of TGFβ mRNA, remained unchanged. TGFβ receptor binding parameters also did not change comparing the 184A1N4 cells and the oncogene transformants. We concluded that differential responsiveness to TGFβ was to a certain extent correlated with expression of the transformed phenotype. However, the differential responsivity could not be explained solely on the basis of altered production of endogenous TGFβ or to differences in TGFβ receptor binding characteristics. Rather, modulation of TGFβ inhibition of these human breast epithelial cells apparently occurs at a level distal to the TGFβ receptor.

In many other systems, such as in human bronchial epithelial cells, growth inhibition by TGFβ is associated with an induction of differentiation (Masui et al., 1986). The effect of TGFβ on the expression of epithelial membrane antigen, a derivative of the breast epithelial specific marker milk fat globule protein (Stampfer and Bartley, 1988), was studied in the 184 cells and sublines. A 10 to 15-fold increase in milk fat globule protein was induced in the normal cells following TGFβ treatment. Full malignant transformation with SV40T and V-ras' did not significantly compromise the TGFβ induction of this differentiation antigen (Walker-Jones et al., 1989). Sodium butyrate, another differentiation-inducing agent, was also found to stimulate milk fat globule protein expression. Thus, the molecular pathway for the growth inhibitory action of TGFβ on the human mammary epithelial cell lines is apparently separable from induction of cellular differentiation, at least as measured by the breast epithelium specific marker milk fat globule protein.

It has been postulated that in fibroblasts, the mitogenic action of TGFβ is coupled to, or mediated by, an induction of expression of c-sis, the platelet-derived growth factor B-chain (Leof et al., 1987). We have previously reported the production of both the A and B chains of PDGF by human breast cancer cells (Bronzert et al., 1987). In examining three of the normal human mammary epithelial cell strains, we found that they produced PDGF receptor competing activity in amounts similar to the levels determined in the conditioned media from the human breast cancer cells (Bronzert et al., 1988). RNase protection analysis of total cellular RNA revealed strong expression of the PDGF A chain. Expression of the PDGF B chain was reversibly induced by TGFβ treatment, while PDGF A chain mRNA was not affected. At this point, the mechanism(s) behind this induction of PDGF B chain associated with both growth inhibition by TGFβ in the human mammary epithelial cells, and growth stimulation in fibroblasts, remain(s)
to be determined.

CONCLUSIONS

From our studies of normal and oncogene transformed human mammary epithelial cells, we can conclude that some of the original hypotheses about mechanisms for malignant transformation, based on studies using fibroblasts, may need to be revised with respect to mammary carcinogenesis. TGF α, initially thought to be directly associated with the transformed state, may be more tightly coupled to cellular proliferation, regardless of the state of transformation. This is also supported by the finding of immunoreactive TGF α and TGF α mRNA in benign human breast lesions (Perroteau et al., 1986; Travers et al., 1988). Likewise, in chemically induced rat mammary adenocarcinomas, the primary lesions were found to express TGF α whereas the serially transplantable, more progressed, carcinomas expressed little or no TGF α (Liu et al., 1987). This lends additional support to the contention that enhanced TGF α production per se can not solely account for the transformed phenotype in mammary malignancies. However, the TGF α-EGF receptor system may be critical in driving proliferation of early, well differentiated malignant lesions. Similar conclusions are drawn in a recent study of mouse epidermal or papilloma cells expressing a human TGF α cDNA in skin grafts on mice. TGF α produced by either tumor cells or adjoining normal cells could stimulate tumor growth, but TGF α could not directly influence tumor progression (Finzi et al., 1988).

The notion that neoplastic proliferation involves escape from normal growth regulation such as inhibition by TGF β is, at least partially, supported by our findings that the most fully transformed, tumorigenic member of the 184-derived cells are also least responsive to TGF β. However, clearly the presence of activated oncogenes in these cells cause more profound alterations in cell growth control than can be entirely explained by altered sensitivity to, or production of TGF β or TGF α.

Similarly, since oncogenic transformation only caused apparently minor changes in EGF receptor expression and no differences in TGF β receptor expression in these human mammary epithelial cells, we can conclude that modulation of cellular responsiveness to these growth factors can occur at a level beyond direct ligand-receptor interactions. Many questions regarding receptor functionality and second-messenger mechanisms have not yet been addressed in our studies of these cells. Taken together, then, we can clearly observe the effects of malignant transformation on cellular responsiveness to two of three major growth regulatory peptides, TGF α, MDGF1 and TGF β, in a model system of normal, immortalized, and oncogene transformed human mammary epithelial cells. It is apparent that alterations of cellular responsiveness are effects of perturbations of growth control at a level beyond alterations in TGF α or TGF β production or cell surface receptors. These alterations encompass sensitization to transforming effects of growth factors and complete replacement of the growth factor pathway with an oncogenic mechanism.

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