Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17β-estradiol or v-Ha-*ras* oncogene

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ABSTRACT   The MCF-7 human breast cancer cell line responds to estrogen stimulation *in vitro* by increased secretion of growth factors and proliferation and *in vivo* by tumor formation in the nude mouse. To test a possible role of growth factor secretion in expression of the tumorigenic phenotype, we stably transfected MCF-7 cells with the v-Ha-*ras* oncogene to produce the MCF-7ras cell line. The MCF-7ras cell line was tumorigenic in the absence of estrogens and secreted 3- to 5-fold elevated levels of a high molecular weight form of a type α transforming growth factor-like growth factor, type β transforming growth factor, and insulin-like growth factor I. MCF-7ras cells, in contrast to MCF-7, were less sensitive to further growth stimulation by estrogen, type α transforming growth factor, and insulin-like growth factor I and showed little change in receptor levels for these hormones. Conditioned medium from MCF-7ras cells as well as two of its component growth factors (insulin-like growth factor I and type α transforming growth factor) replaced estrogen in stimulating MCF-7 colony formation *in vitro*. A coordinate increase in growth factor secretion by human breast cancer may contribute to its escape from estrogen dependence.

Human breast cancers are heterogeneous in their expression of receptors and responsiveness to 17β-estradiol. Clinically, human breast cancer may often be 17β-estradiol-responsive initially, but later may progress to a more aggressive, hormone-independent form (1). Both hormone-responsive and hormone-independent tumor types may be studied *in vitro* using a variety of human breast cancer cell lines of both phenotypes (2).

The MCF-7 cell line, originally derived from the pleural metastases of a patient with adenocarcinoma of the breast (2), contains specific 17β-estradiol receptors and exhibits obligate 17β-estradiol-dependence for tumor formation in the athymic (nude) mouse (3). MCF-7 cells and other estrogen receptor-containing cell lines respond to 17β-estradiol treatment *in vitro* with an increased growth rate and with secretion of growth factor activities (4), including a 30-kDa peptide whose activity resembles type α transforming growth factor (TGF-α) (5–7). Type β transforming growth factor (TGF-β), insulin-like growth factor I, and a platelet-derived growth factor (PDGF)-related polypeptide are also secreted. The fact that epidermal growth factor (EGF) and IGF-I are growth promoting for MCF-7 and some other human breast cancer cell lines (8–10) has led us to believe that TGF-α and IGF-I might be autocrine growth factors. TGF-β, found in platelets and usually secreted by cultured cells in inactive form, is growth inhibitory for receptor-containing breast cancer cell lines (11, 12, 41) while PDGF is not known to be mitogenic for breast cancer. PDGF and TGF-β may stimulate stromal proliferation around the tumor *in vivo*, acting as supportive, paracrine hormones (13).

In an effort to understand more fully the events leading to enhanced growth factor production and tumorigenesis, we have established and described a clone of MCF-7 cells, MCF-7ras, stably transfected by the v-Ha-*ras* oncogene in the presence of a selectable bacterial gene marker *Ecogpt* (14). We also prepared a control transfectant line MCF-7gpt containing only the selectable marker. This latter cell line was quite similar to wild-type MCF-7 in those phenotypic markers examined (growth rate, its 17β-estradiol responsivity *in vitro* and *in vivo*, major intracellular and secreted proteins, responsiveness to antiestrogens, and presence of receptors for estrogen, EGF, and IGF-I). The MCF-7ras cell line retained the ability to respond to 17β-estradiol exposure as measured by induction of progesterone receptor, but had severely blunted growth responses to 17β-estradiol and antiestrogen *in vitro*. In addition the cell line had become estrogen-independent for tumorigenicity *in vivo*. Because we had shown that 17β-estradiol stimulation of MCF-7 cells was required for tumorigenicity *in vivo* and was accompanied by enhanced growth factor secretion (6, 7), we examined whether the MCF-7ras cells secreted activated growth factor in conjunction with their autonomous tumorigenic capacity and whether this growth factor secretion was responsible for tumor growth.

EXPERIMENTAL METHODS

Cell Culture. MCF-7 cells (2) were originally obtained from Marvin Rich, Michigan Cancer Foundation, Detroit, MI. Cells were cultured in 10% (vol/vol) fetal calf serum or in 5% (vol/vol) sulfatase and charcoal-treated calf serum in phenol red-containing improved minimum essential medium (IMEM) (GIBCO) as described (6, 7). A late passage (200) clone of MCF-7 cells stably cotransfected with the transforming DNA from Harvey murine sarcoma virus and the selectable marker pSV2gpt were also used (MCF-7ras) (14). A paired control line transfected only with pSV2gpt (MCF-7gpt) was used as a control for all MCF-7ras studies. For 17β-estradiol-treatment studies, wild-type MCF-7 cells were treated with 1 nM 17β-estradiol or the ethanol vehicle for 4 days (6, 7). DNA was routinely assayed in cell sonicates by a fluorometric assay (15). The 48-hr conditioned medium (CM) collections were carried out, and CM was concentrated and extracted with acid as described (7). Anchorage-independent growth of rat NRK cells, a "transforming growth" assay was carried out as described by Delarco *et al.* (16). CM from Moloney murine sarcoma virus-transformed 3T3 cells (MoMSV-3T3) (from American Type Culture Collection) was routinely used as a positive control.

Growth Factor Assays. The transforming growth assay (16) used was sensitized for the detection of TGF-α-like activity.

Abbreviations: TGF-α, type α transforming growth factor; TGF-β, type β transforming growth factor; IGF-I, insulin-like growth factor I; PDGF, platelet-derived growth factor; CM, conditioned medium; EGF, epidermal growth factor.

Serum containing a high level of TGF-β activity was used. TGF-α-like activity was assayed using ^{125}I-labeled EGF (from mouse, Bethesda Research Laboratories) (17), and the competitive, specific radioreceptor assay (A431 carcinoma cells) of Delarco *et al.* (16). TGF-β was also assayed by a competitive, specific radioreceptor assay (18) in direct concentrates of CM in serial dilutions. Acid extractions were not used to directly study biologically active TGF-β (41). For radioreceptor assay, authentic human platelet-derived ^{125}I-labeled TGF-β and nonradioactive TGF-β (a gift from R. Assoian, A. Roberts, and M. Sporn, National Institutes of Health) were used (19). TGF-β was iodinated (18) by a modified chloramine-T procedure to 2.9 μCi/pmol (1 Ci = 37 GBq). A549 human lung carcinoma cells (a gift from L. Wakefield and M. Sporn), a cell line with high-affinity TGF-β receptors (18), were used for the radioreceptor assay. IGF-I was assayed by RIA in serial dilutions of CM using pure iodinated human IGF-I standard (from Amgen, Thousand Oaks, CA) (85–115 μCi/μg by chloramine-T) (16). A specific antiserum (generously provided by J. J. Van Wyk to the National Pituitary Agency, Bethesda, MD) was used at 1:14,000 dilution with 1:350 dilution of Pansorbin (Calbiochem) for precipitation. Media were acid/ethanol extracted (8) prior to assay. A "competence" activity assay (20) was carried out to screen for PDGF activity. NIH 3T3 cells were used in an assay as described (20) using [^{14}C]thymidine (0.5 μCi/ml, New England Nuclear). Fibroblast growth factor (200 ng/ml, 80% purified factor; Bethesda Research Laboratories) and PDGF (200 ng/ml, 50,000 units/mg; Biomedical Technologies, Norwood, MA) were used as positive controls. For these experiments CM was generally prepared by culturing cells in 0.5% platelet-poor plasma and then concentrating 10-fold by Amicon filtration (see above). CM from NIH 3T3 cells was used as a negative control, and CM from simian sarcoma virus-transformed 3T3 (SSV-3T3) cells (a gift from Mark Israel, National Institutes of Health) and MoMSV-3T3 cells were positive controls. Growth factor measurements were sometimes normalized for DNA content of producer cell monolayer or for bulk secreted protein (dpm of [^{3}H]leucine) (6). Sometimes ratios were taken between 17β-estradiol- or v-Ha-*ras*-stimulated values and appropriate controls. Data are always representative of at least three independent experiments.

Receptor Measurements. EGF receptor was determined using human EGF (Amgen) iodinated by chloramine-T (17). Binding assays and Scatchard analysis were carried out as described (21). IGF-I receptor was determined using human IGF-I (Amgen) iodinated by chloramine-T (16). Binding assays and Scatchard analysis were carried out as described (9). TGF-β receptor was determined using human platelet-derived TGF-β. Binding assays were carried out as described (19).

Colony Stimulation Assays for Growth Factor Activities. To determine if v-Ha-*ras* transfection of MCF-7 cells resulted in enhancement of diffusible mitogenic activities, a coculture procedure was developed. Four compartment, 100-mm diameter culture dishes (Falcon x-plate #1009) were coated with poly(D-lysine) (Sigma) prior to use (22). MCF-7ras or MCF-7gpt were then each plated, increasing in density up to full confluency in two adjacent compartments of culture dishes, and acted as conditioner or feeder cultures in the experiment. One day after plating, the medium was removed and vertical openings were made in plate barriers (2 mm wide and to within 2 mm of the culture dish surface with a heated metal spatula, without allowing cell monolayers to dry). Two openings were made per plate, each between feeder and indicator compartments. Then 5 ml of IMEM/5% (vol/vol) charcoal-treated calf serum was added per compartment for 24 hr, and 400 MCF-7 cells were seeded in the indicator compartment. After 8 days, MCF-7 colonies ($\geq$50 cells) were
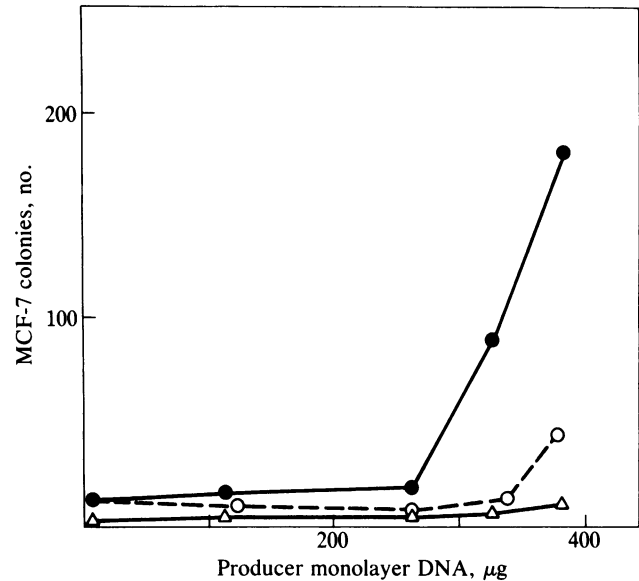
counted after fixation with 90% (vol/vol) ethanol and staining with naphthyl blue black in 5% (vol/vol) acetic acid.

For growth factor studies, MCF-7gpt or MCF-7ras cells were plated in 6-well, 35-mm dishes containing various concentrations of human EGF (Amgen), IGF-I (Amgen), or TGF-α (a gift from Ryk Derynck, Genentech Corp., San Francisco) in IMEM/5% (vol/vol) charcoal-treated calf serum. Other experiments used 1 nM 17β-estradiol. Colonies were counted after 8 days as above.

RESULTS

CM from MCF-7ras Cells: A Mitogen for MCF-7 Cells. Initial experiments tested whether a diffusible activity from MCF-7ras cells was capable of stimulating the growth of wild-type MCF-7 cells. In CM cross-feeding experiments (Fig. 1), we were able to show that MCF-7ras cells secreted more than 3- to 4-fold of a diffusible mitogen than MCF-7gpt

Fig. 1. Effect of MCF-7ras CM on colony formation of MCF-7 cells. MCF-7ras ($\bullet$) or MCF-7gpt ($\circ$) CM cross-feeding was carried out. MCF-7 indicator colonies formed in the continuous presence of medium from feeder monolayers of MCF-7ras, of MCF-7gpt cells or of no cells ($\triangle$) were counted, and data were normalized for DNA of producer cell monolayers. Points are averages of duplicate determinations and are representative of two independent experiments.

Table 1. Detection of NRK transforming activity in CM

Cells	NRK transforming activity in CM		
	Colonies, no. per ml	Colonies, no. per mg of DNA	Colonies, no. $\times$ 10^{-3} per dpm of [^{3}H]leucine-labeled secreted protein
MCF-7gpt	18.3 $\pm$ 4.2	360 $\pm$ 80	18.5 $\pm$ 4.1
MCF-7ras	82.1 $\pm$ 14.6*	1368 $\pm$ 243*	74.6 $\pm$ 13.2*
MoMSV-3T3	340 $\pm$ 40	11,525 $\pm$ 1600	113 $\pm$ 13.3

Media were prepared from MCF-7gpt, MCF-7ras, and MoMSV-3T3 cultures. After concentration and dialysis against fresh IMDM, NRK transforming activity was directly determined (with 4.8-ml equivalents of the CM from MCF-7 cells and 2-ml equivalents of the MoMSV-3T3 CM). The concentration of DNA was determined in CM producer monolayers along with the dpm of [^{3}H]leucine incorporated into secreted protein. NRK transforming activity is expressed per ml of initial volume of medium, per mg of DNA in producer monolayer, and per dpm of secreted protein. Data are the mean $\pm$ SEM of triplicate samples.
*$P < 0.01$ comparing MCF-7gpt to MCF-7ras by Student's *t* test.

Table 2. Elevated levels of IGF-I, TGF-α, and TGF-β in CM for MCF-7ras cells

Cell	TGF-α			TGF-β			IGF-I		
	ng/ml	ng/mg	pg/dpm	pg/ml	pg/mg	fg/dpm	ng/ml	ng/mg	pg/dpm
MCF-7gpt	12 ± 2	218 ± 36	12.3 ± 2.0	10 ± 4.7	181 ± 85	10.2 ± 4.8	4.5 ± 0.5	82 ± 9.1	4.6 ± 0.5
MCF-7ras*	38 ± 5	655 ± 86	39.5 ± 5.1	57 ± 10.3	982 ± 178	59.0 ± 10.6	14.3 ± 1.2	250 ± 20.6	14.8 ± 1.5

CM was collected, concentrated, and assayed for TGF-β directly (radioreceptor assay with A549 carcinoma cells), for TGF-α after acid extraction (radioreceptor assay with A431 carcinoma cells), or for IGF-I after acid/ethanol extraction (RIA with a specific antiserum). Data are expressed as ng of growth factor per ml of CM, ng of growth factor per mg of DNA, or pg of growth factor per dpm of [³H]leucine-labeled secreted protein. DNA was determined in producer monolayers (2.9 mg per flask of MCF-7gpt cells and 2.75 mg per flask of MCF-7ras cells) as was [³H]leucine incorporation into secreted protein (969 dpm/ml of MCF-7gpt CM and 975 dpm/ml of MCF-7ras CM).
*$P < 0.01$, comparing values from MCF-7ras to MCF-7gpt by Student's t test.

cells per unit DNA of the producer monolayer. We analyzed bulk secreted proteins from MCF-7ras and MCF-7gpt cells by NaDodSO₄/gel electrophoresis after labeling with either [³⁵S]cysteine or [³⁵S]methionine, but failed to detect any v-Ha-*ras*-induced proteins. We proceeded to systematically analyze MCF-7ras CM for less abundant proteins. The following polypeptide growth factors have been identified to date in MCF-7 cells are: TGF-α (5–7), TGF-β (7, 23), IGF-I (6, 8), and PDGF (24).

TGF-α. We initially employed a transforming activity assay sensitized for TGF-α-like activity by including high levels of exogenous TGF-β (16). Transforming growth activity of MCF-7ras CM was enhanced 4- to 5-fold more than the control culture (Table 1) after normalization for producer cell DNA or bulk secreted proteins measured by [³H]leucine incorporation into macromolecules. When dilutions of CM were used in the colony assay, it was observed that CM from MCF-7gpt control cells, MCF-7ras and positive control MoMSV-3T3 CM (16) all had parallel dilution profiles (data not shown). Since TGF-α interacts with the EGF receptor, we also assayed EGF receptor binding activity directly using a specific radioreceptor assay. The concentration of TGF-α-like activity was elevated 3-fold in MCF-7ras cells, to approximately the level of the K_d (10) for EGF receptor in MCF-7 (Table 2). MCF-7ras CM was fractionated by gel exclusion chromatography (Fig. 2). Assay of column fractions for EGF–receptor binding activity and NRK transforming activity identified a peak containing a 30-kDa material, which was similar to that obtained with CM from 17β-estradiol-stimulated MCF-7 cells (6, 7). Acid Bio-Gel P-150 chromatography of CM from 17β-estradiol-treated MCF-7 and from MCF-7ras cells also identified the 30-kDa material.

TGF-β. TGF-β, which complements TGF-α in transformation of fibroblasts, was quantitated by a specific radioreceptor assay (19). TGF-β was elevated 5-fold in MCF-7ras cells compared with the MCF-7gpt control cells (Table 2), a concentration reported to affect fibroblast growth (19). Acid Bio-Gel P-60 fractionation of MCF-7ras CM showed that a polypeptide detected by TGF-β radioreceptor assay comigrated with authentic human platelet-derived TGF-β (14 kDa). Intracellular TGF-β was also elevated as shown by immunostaining with an antiserum prepared against an amino-terminal TGF-β peptide (41).

IGF-I. IGF-I has been shown (25) to be required for full expression of the transformed phenotype. IGF-I was assayed by radioimmunoassay in MCF-7 CM using a specific antiserum and authentic IGF-I (Table 2). IGF-I was elevated 3-fold in MCF-7ras cells compared with MCF-7gpt cells, to the reported K_d for the receptor in MCF-7 cells (9). After fractionation of CM on acid Bio-Gel P-60 chromatography IGF-I was detected at 30–40 kDa (and a smaller peak containing 12-kDa material), comigrating in MCF-7ras and MCF-7gpt samples. Authentic ¹²⁵I-labeled IGF-I when added to the sample comigrated with the high molecular weight material. Acid/ethanol extraction of the material in the peak at 30–40 kDa reduced protein binding of both standard and CM-derived activities converting them to 12 kDa, which

comigrated with authentic IGF-I chromatographed in the absence of any CM. Intracellular IGF-I was also elevated in MCF-7ras cells (58.4 ± 5.3 ng/mg of DNA) compared with MCF-7gpt cells (19.2 ± 0.14 ng/mg of DNA).

PDGF Activity. We assayed the MCF-7ras CM for the presence of PDGF competence activity as measured by an NIH 3T3 [³H]thymidine incorporation assay. As the principal competence activity in serum, PDGF acts with TGF-α,
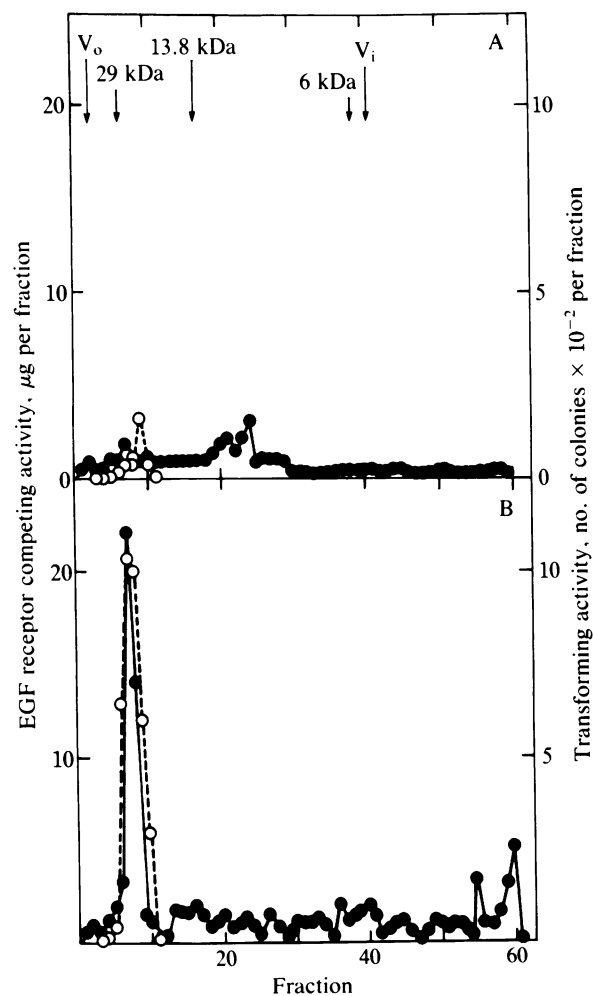

Fig. 2. Fractionation of CM from MCF-7ras (*B*) and MCF-7gpt (*A*) cells; detection of transforming activity (○) and EGF receptor competing activity (●) (TGF-α-like activity). Acid-dialyzed 330× CM concentrate (3 ml) was applied to a Bio-Gel P-60 column (300 ml, bed volume). Fractions (170 drop) were diluted with 1 M acetic acid directly assayed for A_{280}. After lyophilization of fractions and reconstitution with Dulbecco's PBS, pH 7.2 (GIBCO), NRK transforming activity and TGF-α were determined. Molecular size standards are as follows: 29-kDa carbonic anhydrase; 13.8-kDa ribonuclease A; 6-kDa insulin. CM samples were not precisely normalized for producer cell number here; quantitative comparisons between MCF-7gpt and MCR-7ras are in Tables 1 and 2.

TGF-β, and IGF-I to support the maximal expression of transformation of fibroblasts (26). In contrast to TGF-α, TGF-β, and IGF-I, PDGF activity was not further elevated in MCF-7ras cells or in MCF-7 cells stimulated by 17β-estradiol under these conditions (Table 3).

Receptor Levels and Growth Factor Sensitivity. Secretion of three growth factors was elevated by v-Ha-*ras* oncogene transfection into MCF-7 cells in association with development of estrogen and antiestrogen autonomy of the cells (14). MCF-7ras retained substantial amounts of the estrogen receptor (14). In addition, EGF and IGF-I receptors (initially low, but functional) were not significantly down-modulated in MCF-7ras cells (Table 4). The TGF-β receptor was undetectable (less than 500 sites per cell) in the high-affinity (10 pM K_d) range on both MCF-7gpt and MCF-7ras cells. Other clones of MCF-7 cells that contain the TGF-β receptor and that are growth inhibited by TGF-β have been described (11, 41), but in the current study the late passage MCF-7ras and MCF-7gpt clones did not have detectable TGF-β receptors and did not manifest a growth response to TGF-β. As expected, the MCF-7ras cells showed little stimulation of colony formation by 17β-estradiol when compared to MCF-7gpt cells (data not shown). Fig. 3 shows that the growth of MCF-7gpt cells, in contrast to MCF-7ras cells, was stimulated by both TGF-α and EGF. The growth of MCF-7gpt cells, also in contrast to MCF-7ras cells, was stimulated by IGF-I. The concentration of IGF-I that gave the maximal number of colonies was 1 ng/ml; 0.2 ng/ml was half-maximal.

DISCUSSION

Estrogen stimulation and v-Ha-*ras* transfection are independent tumorigenic stimuli for the MCF-7 breast cancer cell line. The tumorigenic signal(s) provided by either of these stimuli are accompanied by the augmented production of growth factors that accumulate to physiologically relevant concentrations. In addition, several tumorigenic, estrogen receptor-negative breast cancer cell lines have been reported that constitutively secrete high levels of TGF-α, TGF-β, and IGF-I (5–8). The data reported here for breast cancer coupled with the findings of others (16, 27–32) provide strong circumstantial evidence that some growth factors are closely associated with the tumorigenic phenotype as achieved by a variety of means. Furthermore, they suggest that enhanced secretion of growth factors may also play a role in a late step in escape of a breast tumor from the requirement for estrogen. In addition, the CM cross-feeding experiment

Table 3. Selectivity of growth factor secretion in MCF-7 cells stimulated by 17β-estradiol or v-Ha-*ras*

Activity	Assay	Fold stimulation over control	
		17β-estradiol	v-Ha-*ras*
TGF-α	NRK colonies	4.8	3.8
	EGF receptor competition	4.4	3.0
TGF-β	Receptor competition	0.4	5.4
IGF-I	Radioimmunoassay	1.0	3.0
PDGF	NIH 3T3 competence activity	0.9	1.2

MCF-7 cells were grown in monolayer culture, serum-free conditioned medium was collected, and growth factor activity was determined. When 17β-estradiol was the stimulus, MCF-7 cells were pretreated 3 days with 1 nM 17β-estradiol in ethanol or ethanol control. When v-Ha-*ras* was the stimulus, the MCF-7gpt control line was compared with the MCF-7ras transfectant line. Data are expressed as fold stimulation over control. The MCF-7 cells treated with ethanol only had very similar levels of secretion of all growth factors compared to MCF-7gpt cells. Data are representative of more than three experiments.

Table 4. Receptors for growth factors

Factor	MCF-7gpt		MCF-7ras	
	Sites per cell, no. $\times$ 10^{-3}	K_d, nM	Sites per cell, no. $\times$ 10^{-3}	K_d, nM
EGF	4.3	1.4	3.6	2.6
IGF-I	11.0	1.1	11.0	0.9
TGF-β	<0.5	—	<0.5	—

Receptors for EGF (TGF-α), IGF-I, and TGF-β were determined using monolayer cultures of cells.

suggests that the development of hormonal autonomy in a portion of a tumor might accelerate the growth of estrogen-dependent cancer in nearby tumor areas, facilitating malignant progression. Consistent with this hypothesis is the finding by Danielpour and Sirbasku (28) that in a murine mammary tumor model system the inoculation of a nude mouse with both hormone-independent and hormone-dependent cell lines confers hormone-independent growth potential to the hormone-dependent cell (28).

A variety of studies in nontransformed rodent fibroblast cell lines have suggested that transformation by v-Ha-*ras* and other retrovirus oncogenes, radiation, and carcinogens is accompanied by secretion of TGF-α and -β and PDGF-like molecules (29–32). TGF-α, TGF-β, IGF-I, and PDGF activities are all required for full manifestation of the transformed,
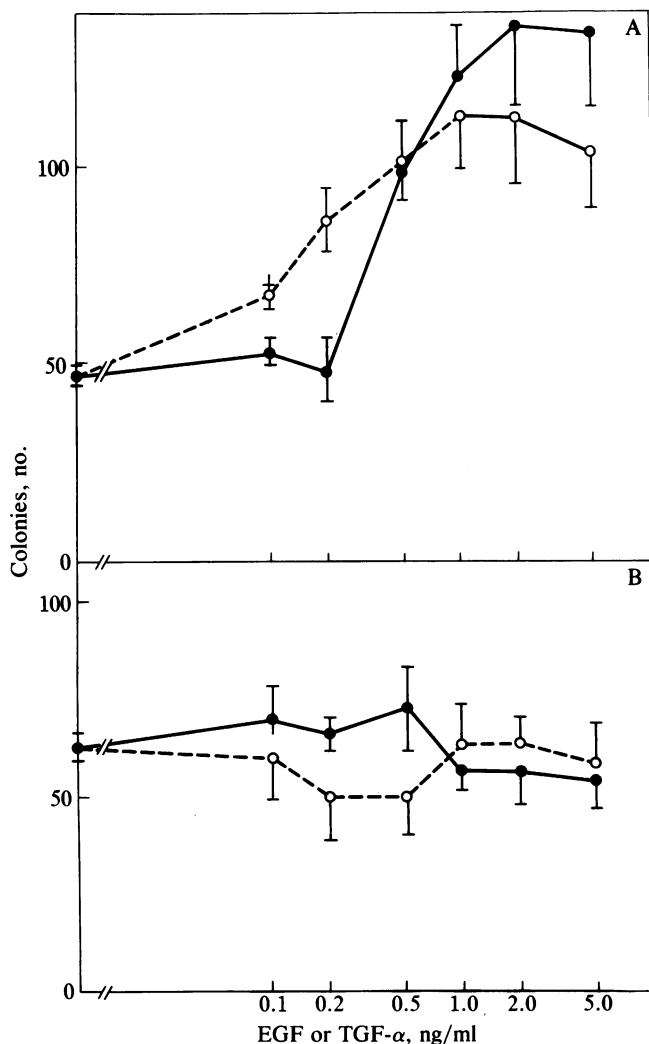

FIG. 3. Effect of EGF (●) and TGF-α (○) on colony formation of MCF-7gpt (A) or MCF-7ras (B) cells. Data are the mean ± SEM of triplicate samples.

anchorage-independent phenotype in nontransformed murine fibroblasts (16, 25, 29–32). In contrast to the fibroblast system, the growth of MCF-7 breast carcinoma cells is only stimulated *in vitro* by IGF-I and TGF-α. TGF-β has been shown to be a hormonally regulated, negative-growth modulator for receptor-containing breast cancer cells (41). The function of TGF-β over expression in the receptor-negative MCF-7ras cells is not known. PDGF and TGF-β may act primarily in a paracrine or some other fashion to help support tumor growth *in vivo* (13). MCF-7 cells, even in the absence of estrogenic or v-Ha-*ras* stimuli, already present the panoply of criteria for *in vitro* transformation including immortality, aneuploidy, anchorage-independent growth, and growth in serum-free medium. MCF-7 cells require estrogen stimulation for tumorigenesis in the nude mouse; this is accompanied by induction of TGF-α (6, 7), proteases (33), and other proteins. Thus, the late step of tumor formation that is induced both by v-Ha-*ras* and by estrogen may be at least partially mediated by TGF-α. Consistent with this hypothesis is that MCF-7 CM and purified EGF stimulated MCF-7 tumor formation in nude mice (34). TGF-α (or EGF) activity may also have much earlier effects in tumorigenesis since Oka and coworkers (35) have shown that removal of the submandibular glands, a major source of EGF, reduces mammary tumorigenesis in susceptible mouse strains. A distinguishing feature of breast cancer growth factor secretion may be the presence of IGF-I. While IGF-like activities are required to support the transformed phenotype in mouse fibroblasts (25), their production by human cancer has been documented so far for only breast cancer and fibrosarcoma (6, 7, 36–38). Interestingly, the MCF-7ras cells bypassed the effects of exogenous EGF, TGF-α, and IGF-I in conjunction with increased growth factor production but without substantial receptor down-regulation.

In summary, we have provided evidence for commonality in the mechanisms of MCF-7 tumorigenesis triggered by hormonal and oncogene signals. Growth factor secretion as triggered by either stimulus could be important in either autocrine or paracrine (i.e., growth or chemotactic effects on the tumor blood vessel supply or connective tissue) modes (39, 40). In addition, induced growth factors could act in conjunction with other tumor products, such as proteases (33), in the promotion of tumor growth.

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