Basic Fibroblast Growth Factor Confers Growth Inhibition and Mitogen-activated Protein Kinase Activation in Human Breast Cancer Cells

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ABSTRACT

The effect of basic fibroblast growth factor (bFGF) on human breast cancer cells was studied in vitro. Exposure to bFGF resulted in significant growth inhibition, decreased DNA synthesis, and accumulation of cells in G0-G1. The IC50 for growth inhibition in MCF-7 cells was 50 pg/ml, and it was abrogated by neutralizing antibodies against bFGF. Inhibition of growth by bFGF was predominant over the growth stimulatory effects of 17β-estradiol, insulin, or epidermal growth factor. Binding and cross-linking studies of 125I-labeled bFGF in intact MCF-7 cells demonstrated 5.2 × 10^3 saturable bFGF binding sites per cell, a dissociation constant of 57 pm, and a M, 142,000 125I-labeled bFGF cross-linked protein. Stimulation of MCF-7 cells with bFGF at concentrations which effect growth inhibition also resulted in activation of p42mapk (ERK2) and p44mapk (ERK1) mitogen-activated protein kinases. These data demonstrate that whereas bFGF inhibits the growth of several breast cancer cell lines, it concomitantly activates ERK1 and ERK2, generally considered to signal mitogenic rather than growth inhibitory responses. Whether there is association between these phenomena remains unknown.

INTRODUCTION

bFGF, also known as FGF-2, is a member of a family of growth and angiogenic factors ubiquitously expressed in normal and malignant tissues (1, 2). bFGF controls the growth of several normal and malignant mesodermal and neuroectoderm-derived cells (3) and malignant epithelial cells (4–7). Its mitogenic activity is mediated via binding to specific high-affinity cell surface receptors and activation of their tyrosine kinase domains (8–12). The downstream signaling was reported to involve the proline-directed serine/threonine MAPKs, ERK1 (p44mapk) and ERK2 (p42mapk; Refs. 8–12). Activation of MAPKs was shown to be required for the mitogenic response of bFGF in fibroblasts (13, 14).

MCF-7 is a hormone-responsive human breast cancer cell line (15). Several hormones and growth factors stimulate MCF-7 growth, including 17β-estradiol, insulin, and EGF (4, 6). In serum-free systems, bFGF was reported to produce a growth stimulatory effect on MCF-7 cells (5, 6). However, in MDA-MB-134, a human breast cancer cell line that overexpresses the FGF receptors 1 and 4, acidic FGF, or bFGF were reported to inhibit cell growth (16). In the present study, we demonstrate that bFGF inhibits the growth of several serum-fed MCF-7 cell sublines as well as other breast cancer cell lines (MDA-MB-453 and T47-D). Exposure of MCF-7 cells to bFGF also activated ERK1 and ERK2, but whether the latter effect was associated with bFGF-induced inhibition of MCF-7 cell growth remains unknown.

MATERIALS AND METHODS

Materials. Human recombinant bFGF (157 amino acids), antihuman bFGF polyclonal IgG, and normal goat IgG were purchased from R&D Systems, Inc. (Minneapolis, MN), Na[125]I and ECL were purchased from Amersham Corp. (Arlington Heights, IL), and DSS was obtained from Pierce Chemical Co. (Rockford, IL). All reagents used for SDS-PAGE were purchased from Bio-Rad (Melville, NY). [methy]-3H]Thymidine (5 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Heat-inactivated FCS was obtained from HyClone Laboratories, Inc. (Logan, UT), and all tissue culture media were purchased from Life Technologies, Inc. (Grand Island, NY). Insulin was obtained from Eli Lilly (Indianapolis, IN). Immobilon P membrane was purchased from Millipore Corp. (Marlborough, MA), anti-MAPK (ERK2) monoclonal antibody.
was purchased at United Biomedical, Inc. (Lake Placid, NY), antiphosphotyrosine monoclonal antibody was purchased from Transduction Laboratories (Lexington, KY), protein G-plus/protein A-agarose was purchased from Oncogene Science (Uniondale, NY). Fetal human recombinant EGF and all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were reagent grade.

**Cell Culture.** MCF-7, T-47D, and MDA-MB-453 cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in phenol red-containing DMEM (high glucose). The medium was supplemented with 2 mM glutamine, 5% heat-inactivated FCS, penicillin (50 units/ml), streptomycin (50 μg/ml), and insulin (10 IU/liter). In some experiments, when the effect of estrogen deprivation on cell growth was studied, the cells were cultured in phenol red-free DMEM supplemented with 5% dextran-coated charcoal-stripped heat-inactivated serum (17). Cell cultures were maintained at 37°C in 5% CO2-humidified incubators. Clonal populations of BAECs were established from the intima of a bovine aorta (18). These cells were cultured in DMEM containing 10% FCS and maintained in 37°C in 10% CO2-humidified incubators.

**Cell Growth Studies.** bFGF stock solutions were dissolved in PBS containing 0.1% human serum albumin, kept frozen at −20°C, and used only once after thawing. MCF-7 monolayers were dispersed with trypsin-EDTA, counted in a Coulter Counter (Coulter Electronic, Inc. Hialeah, FL), and plated in 35-mm dishes, at an initial density of 2 × 104cells/dish, in 2 ml of standard growth medium. The medium was replaced 24 h later with growth medium containing bFGF or the vehicle as described in Fig. 1. The medium was replaced thereafter with fresh medium of the same composition every 2 days.

**Thymidine Incorporation Assay.** Cells (1.6 × 105 cells/well) were incubated in 24-well plates (Falcon, Oxnard, CA) in standard medium for 24 h. The medium was changed to the experimental conditions, and the resulting mitogenic activity was measured by pulsing with [methyl-3H]thymidine. Each well received 1 μCi/well on day 4 for 2 h of incubation. Incorporated thymidine was determined by scintillation spectroscopy of trichloroacetic acid extracts of cells dissolved in 0.1 N NaOH.

**Cell Cycle Analysis.** The effect of bFGF on cell cycle distribution of subconfluent MCF-7 cells was determined by analysis of DNA content using flow cytometry as described previously (19). In short, monolayers were dispersed with trypsin-EDTA and the cells were fixed in 70% ethanol and washed with PBS. Cellular DNA was digested with 160 μg/ml of RNase A. After washing, DNA was stained with 50 μg/ml of propidium iodide. The content of stained DNA was then determined by using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Cell cycle analysis of the DNA histograms was carried out with the Multicycle Computer program (Phoenix Flow Systems, San Diego, CA) developed by P. S. Rabinovitz (University of Washington, Seattle, WA).

**Iodination of bFGF.** Recombinant bFGF was iodinated with chloramine T as described previously (20, 21). Biological activity of 125I-labeled FGF was determined by its ability to stimulate growth of cultured BAECs (3).

**High-Affinity Binding of 125I-labeled bFGF.** Subconfluent cultures (105 MCF-7 cells in 16-mm wells) were pre-cooled to 4°C and washed once with cold PBS. Binding buffer [25 mM HEPES (pH 7.5) and 0.2% gelatin in DMEM] was added to each well followed by addition of 125I-labeled FGF to the desired concentration. Nonspecific binding was determined in the presence of 100-fold excess of unlabeled bFGF. The cells were incubated for 2 h at 4°C. At the end of the incubation, cells were washed twice with PBS supplemented with 0.1% BSA and once with 2 μl NaCl in 20 μl HEPES (pH 7.5) to remove the low-affinity bound bFGF (22). Cells were then lysed in PBS containing 1% Triton X-100, and cell-associated radioactivity was determined in a gamma counter (Gamma 5500; Beckman Instruments, Fullerton, CA).

**Cross-Linking of 125I-labeled bFGF to Its Binding Sites.** Cells were grown to confluence in 10-cm plates and washed twice with cold PBS. The cells were then incubated for 2 h at 4°C in binding buffer containing 125I-labeled bFGF (5 ng/ml). After the incubation, cells were washed with cold PBS supplemented with 0.1% BSA. Bound 125I-labeled bFGF was cross-linked by adding DSS to a final concentration of 0.15 mM for 20 min at room temperature. The reaction was terminated by addition of 1 ml Tris-HCl (pH 7.5) and 150 mM glycine for 5 min followed by a wash with cold PBS. Cells were scraped from the plate, centrifuged, and resuspended in 40–60 μl of lysis buffer (10 mM Tris-HCl (pH 7.0), 0.5% NP40, 0.1 mM EDTA, and 1 mM PMSF) for 10 min at 4°C. After centrifugation at 12,000 rpm in an Eppendorf microcentrifuge for 5 min, the proteins in the supernatant were resolved on 7.5% SDS-polyacrylamide gels. The gels were fixed, dried, and exposed to Kodak X-Omat autoradiography film at −70°C for detection of 125I-labeled bFGF cross-linked peptides.

**Determination of ERK1 and ERK2 Phosphorylation.** Standard culture media were replaced with serum-free DMEM, 2 mM glutamine, and 0.5% BSA fraction V (Calbiochem, La Jolla, CA) 24 h before stimulation with bFGF. Cells were then removed from the plates by a 10–15-min incubation in PBS containing 1 mM EDTA (pH 8.0) at 37°C. Following centrifugation, cells were incubated at 37°C in a shaker bath with the indicated concentrations of bFGF for various time periods. After 10 min of centrifugation at 2000 rpm at 4°C, the cells were prepared for Western blotting or for immunoprecipitation as follows: for Western blotting (23), cells were disrupted by sonication in 200 μl of lysis buffer (20 mM Tris-HCl (pH 7.4), 1 mM EGTA, 50 μM NaVO4, 50 mM NaF, 0.01 unit/ml leupeptin, and 1 mM PMSF). The proteins were resolved on 12.5% SDS-polyacrylamide gels, transferred onto Immobilon P membranes, immunoblotted with antiphosphotyrosine monoclonal antibody, and visualized with the ECL system. The membranes were then stripped with 2% SDS/100 mM 2-mercaptoethanol in 62.5 mM Tris-HCl (pH 6.7), reblotted with anti-ERK2 antibody, and visualized as above. For immunoprecipitation, the cells were lysed in KLB buffer (1% Triton X-100, 0.05% SDS, 10 mM NaH2PO4, and 150 mM NaCl) passing through a 26-gauge needle. The proteins (400 μg/0.5 ml) were heated at 100°C for 10 min, and after cooling were incubated at 4°C with 1 μg of anti-ERK2 monoclonal antibody overnight. The immune complexes were incubated with 15 μl of protein G plus/protein A-agarose for 2 h at 4°C. After washing twice with 1 ml of KLB without SDS and twice with 50 mM Tris (pH 7.5), the immune complexes were resolved on SDS-polyacrylamide gels and immunoblotted as described above.
bFGF. Cells (2 × 10^4/dish) were seeded into 35-mm dishes containing standard (STD) or hormone-deprived growth media (HDM) with or without bFGF. Culture media containing fresh bFGF or control (0.1% albumin) were replaced every other day. Points, means of triplicates; bars, SD.

**Fig. 1** Inhibition of growth of MCF-7 cells by bFGF. Cells (2 × 10^4/dish) were seeded into 35-mm dishes containing standard (STD) or hormone-deprived growth media (HDM) with or without bFGF. Culture media containing fresh bFGF or control (0.1% albumin) were replaced every other day. Points, means of triplicates; bars, SD.

**Determination of ERK1 and ERK2 Enzymatic Activity.** bFGF was added to the tissue culture dishes for 10 min. At the end of the incubation, the cells were washed twice with PBS, scraped into lysis buffer [20 mM Tris (pH 8.0), 40 mM Na_2PO_4, 50 mM NaF, 5 mM MgCl_2, 100 µM Na_3VO_4, 10 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 20 mg/ml leupeptin, 20 mg/ml aprotinin, and 3 mM PMSF], and incubated on ice at 4°C for 10 min. The lysate was centrifuged at 10,000 × g for 10 min and boiled after adding sample buffer to a final concentration of 62.5 mM Tris (pH 6.8), 2.3% SDS, 5 mM EDTA, 10% glycerol, and 100 mM DTT. ERK1 and ERK2 activity was determined by an in-gel kinase assay as described previously (24, 25). Briefly, the proteins were resolved on a 10% SDS-polyacrylamide gel containing 0.5 µg/ml myelin basic protein. The gel was washed for 1 h in each of the following solutions: twice in 20% 2-propanol in 50 mM Tris-HCl (pH 8.0), once in 5 mM 2-mercaptoethanol in 50 mM Tris-HCl (pH 8.0), and twice in 6 M guanidine-HCl. The gel was then washed for 16 h with three changes of 5 mM 2-mercaptoethanol and 0.04% Tween 40 in 50 mM Tris-HCl (pH 8.0). This was followed by incubation with 100 ml of phosphorylation buffer [25 mM HEPES (pH 7.4), 10 mM MnCl_2, and 250 µCi of [γ-^32P]ATP] for 3 h. After incubation, the gel was washed successively with water, 40 mM HEPES (pH 7.4), and 1% sodium PPi in 40 mM HEPES (pH 7.4). The gel was vacuum dried and exposed to autoradiography. Phosphorylated myelin basic proteins associated with proteins in the lysates which comigrated with immunoprecipitated ERK1 and ERK2 were cut, and their associated radioactivity was measured in a scintillation counter.

**Statistical Analysis of Data.** High-affinity binding parameters of ^125_I-labeled bFGF were determined using Scatchard analysis (26). Student’s t test was used to analyze the differences between mean values.

**RESULTS**

**Effect of bFGF on Growth and DNA Synthesis in Human Breast Cancer Cells.** Fig. 1 shows that bFGF inhibits the growth of early passage MCF-7 cells obtained from the American Type Culture Collection. Exposure to bFGF (500 pg/ml) induced a significant growth inhibition of cells cultured in either standard medium (P < 0.001) or in hormone-deprived medium (P < 0.01). Concomitantly, there was a 90% decrease in DNA synthesis, as demonstrated by the use of the [³H]thymidine incorporation assay (Fig. 2). Thymidine uptake was increased in the presence of 17β-estradiol, insulin, or EGF, but costimulation with bFGF abrogated the effects of these factors on DNA synthesis (Fig. 2).

The inhibition of DNA synthesis by bFGF was dose dependent. The IC_{50} in MCF-7 cells was 50 pg/ml, and a maximal effect was observed with concentrations equal to or greater than 250 pg/ml (Fig. 3). The antiproliferative effect of bFGF was also dependent on the duration of exposure. An inhibitory effect was
Growth Inhibition of Breast Cancer Cells by bFGF

The growth inhibition of breast cancer cells by bFGF was assessed by thymidine incorporation after 96 h. Columns, means of quadruplicates; bars, SD.

Fig. 3 Dose-response curve of the inhibition of DNA synthesis by bFGF. MCF-7 cells (1.6 x 10⁵ cells/well) were seeded into 24-well plates and exposed to increasing concentrations of bFGF. DNA synthesis was assessed by thymidine incorporation after 96 h. Columns, means of quadruplicates; bars, SD.

Fig. 4 Reversal of bFGF-induced DNA synthesis inhibition by neutralizing anti-bFGF antibodies. Cells were plated in standard media in 24-well plates. After 24 h, the medium was replaced with standard condition medium (A), bFGF (500 pg/ml, B), anti-bFGF antibodies (25 µg/ml, C), bFGF and anti-bFGF antibodies (D), normal goat IgG (25 µg/ml, E), or bFGF and normal goat IgG (F). Thymidine incorporation was determined after 4 days of exposure as described in "Materials and Methods." Columns, means of quadruplicates; bars, SD.

noticed at 8 h after addition of bFGF (500 pg/ml) and was maximal after 96 h of exposure (data not shown).

The specificity of the bFGF effect was determined using neutralizing anti-bFGF antibodies (Fig. 4). Furthermore, the inhibition of growth by bFGF was reversible, indicating a cytostatic rather than a cytotoxic effect. Trypan blue exclusion, evaluated after 96 h of bFGF exposure, revealed that 95% of the cells were viable (data not shown). Replacement of bFGF-containing medium with standard growth medium resulted in resumption of exponential growth, as demonstrated by an increase in cell number and in DNA synthesis (data not shown).

Table 1 shows the effect of bFGF on proliferation and thymidine incorporation in a variety of breast cancer cells. In addition to MCF-7 sublines from different sources, the estrogen receptor-negative breast cancer line MDA-MB-453 and the estrogen receptor-positive breast cancer cell line T47-D were significantly inhibited by bFGF. BAECs were used as a positive control for cells undergoing growth stimulation by bFGF. In these cells, bFGF induced a 3.8-fold increase in thymidine uptake.

Effect of bFGF on Cell Cycle Distribution. Exposure of proliferating MCF-7 cells to bFGF (1 ng/ml) for 24 h resulted in a significant change in cell cycle distribution compared to controls (Fig. 5). The fraction of cells in G<sub>2</sub>-M increased after exposure to bFGF from 51 to 82%, whereas the fraction of cells in the G<sub>1</sub>-phase decreased from 41% to 14% and in G<sub>2</sub>-M from 9% to 4%. These data indicate that bFGF induces a G<sub>1</sub> block in MCF-7 cells.

Binding Affinity of ¹²⁵I-labeled bFGF to Intact MCF-7 Cells. High-affinity binding of ¹²⁵I-labeled bFGF to intact cells at 4°C disclosed the existence of saturable binding sites (Fig. 6A). Scatchard analysis of the binding (Fig. 6A, inset) revealed 5.2 x 10⁵ high-affinity binding sites/cell, with a dissociation constant of 57 pM. Receptor cross-linking to ¹²⁵I-labeled bFGF revealed a M<sub>r</sub> 160,000 protein (Fig. 6B). Assuming that the molecular weight of the cross-linked bFGF peptide is 18,000, the corresponding molecular weight of the cross-linked receptor would be approximately 142,000. This is similar to data on the molecular weight of the FGF receptor obtained by several investigators in other systems (8, 12, 27).

Effect of bFGF on ERK1 and ERK2 Activation. Activation of ERK1 and ERK2 leads to tyrosine and threonine phosphorylation in target proteins (28). Activation of ERK1 and ERK2 by bFGF was evaluated by the tyrosine phosphorylation of ERK1 and ERK2 and by an in-gel kinase assay. Fig. 7 shows that exposure of MCF-7 cells to bFGF resulted in ERK1 and ERK2 phosphorylation. Western blotting analysis of the cell lysates showed that M, 42,000 and 44,000 phosphoproteins that comigrated with ERK2 and ERK1 were tyrosine phosphorylated by bFGF in a dose-dependent manner (data not shown). Immunoprecipitation of MAPKs with anti-ERK2 monoclonal antibody and immunoblotting with anti-phosphotyrosine monoclonal antibodies confirmed a dose-dependent phosphorylation of ERKs by bFGF (Fig. 7). Maximal phosphorylation was observed with 1 ng/ml of bFGF, which increased the activity of ERK1 and ERK2 by 2.1- and 6.2-fold above control, respectively. Incubation with a higher concentration of bFGF (10 ng/ml) resulted in a similar (2.3-fold) increase in ERK1 activity and a 9.9-fold increase in ERK-2 activity. Fig. 8 demonstrates

### Table 1 Effect of bFGF (500 pg/ml) on proliferation and thymidine incorporation of various breast cancer cell lines and BAECs

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Thymidine incorporation (% of control)</th>
<th>Cell count on day 7 (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 (ATCC)</td>
<td>15 ± 4</td>
<td>7.7 ± 4</td>
</tr>
<tr>
<td>MCF-7R</td>
<td>47 ± 7</td>
<td>56 ± 1</td>
</tr>
<tr>
<td>MCF-7L</td>
<td>64 ± 10</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>MCF-7P</td>
<td>64 ± 6</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>T47D</td>
<td>83 ± 13</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>61 ± 7</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>BAEC</td>
<td>385 ± 11</td>
<td>469 ± 17</td>
</tr>
</tbody>
</table>

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Fig. 5 The effect of bFGF on the cell cycle phase distribution of MCF-7 cells. A, cell cycle distribution of proliferating cells in standard growth conditions. B, 24 h after exposure of MCF-7 cells to bFGF (1 ng/ml). DNA content was measured by staining the DNA with propidium iodide (PI).

Fig. 6 Concentration dependence of $^{125}$I-labeled bFGF binding to cells and cross-linking of $^{125}$I-labeled bFGF to cell surface receptors. A, confluent MCF-7 cells were incubated with various concentrations of $^{125}$I-labeled bFGF as described in “Materials and Methods.” Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled bFGF. The amount of specifically bound $^{125}$I-labeled bFGF was then determined. Inset, data plotted according to Scatchard (26). Points, means of triplicate determinations and the SD did not exceed ±10%. B, confluent cultures of MCF-7 cells were incubated with $^{125}$I-labeled bFGF (5 ng/ml) in the absence (Lane a) or presence (Lane b) of a 100-fold excess of unlabeled bFGF at 4°C for 2 h. The bFGF receptor complexes were cross-linked by adding DSS as described in “Materials and Methods” and were analyzed using SDS-PAGE. The resulting autoradiogram (4-day exposure) is shown. Left, molecular weights in kDa.

the effect of duration of exposure of bFGF (10 ng/ml) on ERKs phosphorylation. Maximal effect was observed after 5 min of bFGF exposure and after 4 h it returned to a near baseline level. ERK1 and ERK2 activities were similar in cells grown in standard media and in cells preincubated in serum-free media 24 h prior to stimulation with bFGF.

Exposure of MCF-7 cells to insulin (1–100 μg/ml) also induced ERK phosphorylation, but similar exposure to 17β-estradiol (0.1–10 nm) had not affected ERK1 and ERK2 phosphorylation (data not shown). Coincubation of bFGF (10 ng/ml) with either 17β-estradiol or insulin yielded the same level of ERK phosphorylation as observed with bFGF alone (data not shown).

DISCUSSION

This study demonstrates that bFGF is a potent growth inhibitor of the hormone-responsive human breast cancer cell line MCF-7. bFGF induced a G1 block, and growth inhibition by bFGF was predominant over the mitogenic effect of estrogen, insulin, or EGF. The negative growth regulation conferred by bFGF was dose and time dependent and was reversible upon...
removal of bFGF from the culture medium. The specificity of the bFGF antiproliferative effect was demonstrated by its neutralization with a specific anti-bFGF antibody.

The marked inhibitory effect of bFGF on breast cancer cells contrasts the reported mitogenic effect of bFGF on a large number of normal and transformed cells (1-3). Consistent with our observations, however, Schweigerer et al. (29) reported that bFGF purified from SK-ES1 Ewing sarcoma cells inhibited SK-ES1 cell growth, although it stimulated proliferation of other cells. In addition, Zhou and Serrero (30) reported that bFGF inhibited the proliferation of a highly tumorigenic insulin-independent teratoma-derived cell line. Unlike the reversibility of the growth-negative effect observed in our cells and in SK-ES1 cells (29), the bFGF inhibitory effect in the teratoma-derived cell line was irreversible, although the cell remained viable, suggesting the induction of terminal differentiation.

Several studies reported that bFGF may stimulate or inhibit the growth of the same cell type, depending on the culture conditions under which the experiments were carried out. Karey and Sirbasaku (6) reported that under serum-deprived conditions, bFGF alone stimulated the growth of MCF-7 cells, whereas in combination with insulin, insulin-like growth factor I, or EGF, it inhibited cell proliferation relative to the growth in the absence of bFGF. Stewart et al. (4) reported that under estrogen-deprived conditions, bFGF alone had a minimal growth stimulation effect on MCF-7 cells, but increased proliferation was observed when bFGF was given in combination with estradiol, beyond the proliferative effect of estradiol alone. When these cells lines were grown in the presence of estradiol and insulin-like growth factor I, however, bFGF induced a significant dose-dependent growth inhibition. These observations are consistent with our data that demonstrated a maximal growth inhibitory effect by bFGF in the presence of estradiol and insulin, and a lower growth inhibitory effect in hormone-deprived media (Fig. 1). Furthermore, the growth of MCF-7L, MCF-7P, and MCF-7R sublines was significantly inhibited by bFGF when cultured in standard media (Table 1). The same cells, however, were reported to show increased thymidine uptake when stimulated by bFGF under serum and hormone deprivation (5, 7).

Taken together, these data indicate that bFGF is a pleiotropic biological activator capable of inducing mutually exclusive cellular functions (i.e., proliferation and an antiproliferative effect) under different conditions. The fact that distinctly opposing end results are observed in response to bFGF raises the possibility that the pattern of bFGF signal transmission and the ultimate outcome may be subject to transmodulatory regulation through signaling systems concomitantly activated by other cellular stimulators such as estradiol and insulin. Transmodulatory interactions between signaling systems, leading to an outcome which is different from that expected of a single stimulating agent, have been reported for activation of T-helper cells by interleukin 1 and the T-cell receptor, the reciprocal regulation of apoptosis via ceramide and PKC (31-33), and the ability of 1,2-diacylglycerol to convert an apoptotic response to ceramide into a proliferative response in T Jurkat cells (34). Coordinate signaling resulting from costimulation by bFGF, estradiol, insulin, and possibly other serum factors may explain the differences in response of breast tumor cells to bFGF under various culture conditions. This hypothesis would suggest that the outcome of bFGF stimulation may depend not only on specific phenotypic features of MCF-7 cells, but also on variations of the

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Fig. 7 Effect of increasing concentrations of bFGF on MAPK tyrosine phosphorylation. MCF-7 cells were exposed to increasing concentrations (0.1-100 ng/ml) of bFGF. Phorbol-2-myristate 13-acetate (PMA; 100 nm) was used as a positive control. After 15 min of exposure to bFGF, the cells were lysed and immunoprecipitated by anti-ERK2 monoclonal antibody and then electrophoresed, immunoblotted with an antiphosphotyrosine monoclonal antibody, and visualized with an ECL system. Relative intensity of signals was determined by a scanning densitometer. Molecular weights are indicated in kDa.

Fig. 8 Effect of duration of bFGF exposure on MAPK phosphorylation. Cells were exposed to bFGF (10 ng/ml) for the indicated time intervals. Then cells were lysed and immunoprecipitated by anti-ERK2 monoclonal antibody, electrophoresed, and immunoblotted with an antiphosphotyrosine monoclonal antibody. Signals were visualized with an ECL system. Relative intensity of signals was determined by a scanning densitometer. Molecular weights are indicated in kDa.
microenvironment in which the bFGF signal is generated. This concept may have important implications for understanding the clinical effects of biological response modifiers and other therapeutic agents when used in combinations in the management of breast cancer.

The observation that MAPK is activated in MCF-7 cells under conditions in which bFGF signals an antiproliferative effect is inconsistent with reported observations on the involvement of MAPK in mediating the effects of bFGF (13, 14). The FGF receptors engage in at least two distinct signal transduction pathways. Autophosphorylated tyrosine residues of activated bFGF receptors bind and transactivate the γ isoform of phospholipase C to initiate turnover of membrane phosphoinositides, generate 1,2-diacylglycerol, and activate PKC (35, 36). This signaling pathway is apparently not essential for regulating the mitogenic effect of bFGF, since a point mutation at the tyrosine residue 766 of the receptor was found to inhibit the phospholipase Cγ-PKC cascade, but had no effect on the mitogenic response (37, 38). An alternative pathway was shown to involve activation of Ras and Raf1, which initiate signaling through the MAPK cascade to induce transcription of early response genes and progression through G1-S-phase and G2-M checkpoints of the cell cycle and cell division (39). Furthermore, in some cells PKC was found to mediate the mitogenic effect of FGF (40), and this effect was shown to be mediated via the Raf1-MAPK cascade (41). Whether MAPK is causatively associated in MCF-7 growth inhibition by bFGF remains unknown. More details on the signaling initiated by bFGF in MCF-7 cells will be required to delineate which elements are associated downstream with the antiproliferative effect.

Our study demonstrates that bFGF, while acting as an inhibitory growth factor in human breast cancer cells, also activates ERK1 and ERK2. The data do not, however, provide a cause-and-effect association between these effects. Our data also show that the cytostatic effect of bFGF is reversible and can be modulated by other biological response modifiers that affect MCF-7 cells. The demonstration that biological agents may have opposing effects on human breast tumor cells when coexposed to other agents may have important implications in the design of combined agent therapy in the management of breast cancer.

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Basic fibroblast growth factor confers growth inhibition and mitogen-activated protein kinase activation in human breast cancer cells.

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