Biochemical and Biophysical Research Communications **277,** 255–260 (2000)
doi:10.1006/bbrc.2000.3655, available online at http://www.idealibrary.com on IDEAL®

Intracellular FGF-2 Promotes Differentiation in T-47D Breast Cancer Cells

Reju M. Korah, Vilayvanh Sysounthone, Elizabeth Scheff, and Robert Wieder[1]
Division of Medical Oncology/Hematology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103

Received September 7, 2000

To test the implicated role of basic fibroblast growth factor (bFGF; FGF-2) in promoting differentiation in breast cancer, we enforced the expression of FGF-2 in T-47D breast cancer cells. Expression of FGF-2 conferred an overall less malignant phenotype to T-47D cells as revealed by their reduced proliferative response, impaired capacity for anchorage-independent growth, and invasion through Matrigel. To understand one candidate mechanism for the intracellular FGF-2-mediated anti-invasive effect, we examined the effect of FGF-2 on T-47D cell motility. Addition of recombinant FGF-2 to the growth medium markedly enhanced cell motility while constitutive expression of intracellular FGF-2 significantly inhibited the migratory potential of T-47D cells in a dominant manner. FGF-2-expressing T-47D cells also formed relatively defined branching structures in Matrigel matrices, a characteristic phenotype of differentiation in breast cancer cells. These data suggest a potential role for FGF-2 in promoting functional differentiation of breast epithelial cells.

Key Words: **FGF-2; breast cancer; T-47D cells; migration; proliferation; differentiation; branching morphogenesis.**

Basic fibroblast growth factor (bFGF; FGF-2) belongs to the family of 20 fibroblast growth factors, many of which promote growth, motility and transformation in a variety of cell types, including fibroblasts and endothelial cells (1–3). Cellular responses to FGF-2 are mediated through a dual receptor system consisting of high affinity transmembrane tyrosine kinase receptors (fibroblast growth factor receptors 1 to 4) and low affinity cell surface heparan sulfate proteoglycans (4, 5). Like acidic fibroblast growth factor (FGF-1), FGF-2 does not contain an export signal sequence but nevertheless, is released from cells and can act in an autocrine or paracrine manner. Recent data suggested that intracellular FGF-2 may have unique functional roles that are independent of classical receptor-mediated functions (6).

Five species of FGF-2 with molecular weights of 18, 22, 22.5, 24, and 34 kDa are synthesized by cells as a result of alternate initiation of translation of the same mRNA either at an AUG codon or at four upstream in-frame CUG codons (7–9). The 18-kDa FGF-2 isoforms are predominantly localized in the cytoplasm and are most frequently found exported from the cells (10). High molecular weight (HMW) isoforms (22, 22.5, 24, and 34 kDa) on the other hand, contain nuclear localization sequences and are predominantly found localized in the nucleus (9, 11). The relative amounts of the individual isoforms differ greatly among various cell types and tissues during development (7, 8, 12). Varied expression levels as well as release patterns of the different isoforms of FGF-2 and their developmentally regulated expression profiles suggest opportunities for isoform-specific roles, probably with cell type specific phenotypic outcomes. Constitutive expression of individual FGF-2 isoforms in NIH-3T3 cells in fact support this suggestion by promoting shared as well as distinct signaling events and isoform-specific phenotypes (10, 13).

Contrary to its role in promoting transformation and motility in cells of mesodermal and neuroectodermal origin, various *in vivo* and *in vitro* observations suggest that FGF-2 promotes a more differentiated phenotype in breast epithelial cells (14). In infiltrating ductal carcinomas, FGF-2 staining is detected only in basement membranes, not in tumor cells (15). Similarly, significantly less FGF-2 message is present in breast cancer biopsies than in nonmalignant biopsies (14). Moreover, among mammary epithelium-derived cell lines, FGF-2 expression is found only in normal cells while it is undetectable in most malignant cell lines, including MCF-7, T-47D, ZR-75-1, and MDA-MB-231 (14). Recently it was shown that FGF-2 could induce

[1] To whom correspondence should be addressed at UMDNJ-New Jersey Medical School, MSB I-594, 185 South Orange Avenue, Newark, NJ 07103. Fax: 973-972-2384. E-mail: wiederro@umdnj.edu.

0006-291X/00 $35.00

specific growth arrest in breast cancer cells through classical receptor mediated signaling (16). We have also shown that enforced expression of FGF-2 in the highly malignant breast cancer cell line MDA-MB-231 reversed their malignant behavior significantly (6). None of the observed effects in these cells were recapitulated by extracellular FGF-2, however. Hence, the induction of a less malignant phenotype by expression of cytoplasm-localizing FGF-2 suggested the possibility of a novel cytoplasmic effect for FGF-2 in breast cancer cells, potentially unique to breast epithelial cells. In the present study, we have addressed this provocative concept in the context of an implicated differentiation-promoting role for FGF-2. We demonstrate here, for the first time, that constitutive expression of FGF-2 promoted differentiation in T-47D breast cancer cells. Expression of FGF-2 not only resulted in significant reduction in their malignant properties, but also promoted branching morphogenesis, a characteristic response of normal breast epithelial cells when induced to undergo functional differentiation.

MATERIALS AND METHODS

Cell culture, FGF-2 expression, and Western blots. T-47D human mammary epithelial cells (purchased from American Type Culture Collection-ATCC, Rockville, MD) were cultured in standard medium, i.e., Dulbecco's modified Eagle's medium (DME) containing 10% fetal calf serum, 2 mM L-glutamine and penicillin 50 units/ml/ streptomycin 50 μg/ml (Gemini Bio-Products, Calabassas, CA). Construction of pCI-neo (Promega, Madison, WI) expression vectors carrying sequences for various FGF-2 isoforms, transfections and selections were carried out as described before (6). The FGF-2 cDNA constructs were sequenced and found to have no mutations (6). Cell populations expressing 18 kD FGF-2 (ΔA) or the 18, 22, 22.5 and 24 kD isoforms together (FGF) were isolated along with vector transfected controls (Neo) and were designated as T-47D/ΔA, T-47D/FGF and T-47D/Neo, respectively. Expressions of various FGF-2 proteins was verified by Western immunoblots as before (6) using monoclonal anti-FGF-2 antibody (Oncogene Science, Cambridge, MA).

Cell growth kinetics and growth in soft agar. Cells were trypsinized (Trypsin 0.05%/EDTA 0.53 mM; Life Technologies Inc., Gaithersburg, MD) and incubated on 100-mm tissue culture dishes at an initial density of 2.5×10^5 cells/dish in 10 ml standard medium, with and without 1.0 ng/ml recombinant human FGF-2 (rhFGF-2; R&D Systems, Minneapolis, MN). Viable cell numbers were determined manually by 0.2% trypan blue exclusion assay (6, 16). Colony formation efficiency in soft agar was determined in 0.3% bacto agar (Difco Labs, Detroit, MI), 0.67 DMEM, 6.7% FCS overlaying a preformed 0.6% agar layer. Thirty-five-millimeter diameter plates containing 5000 cells in agar were incubated at 37°C in 5% CO_2, and colonies containing greater than 30 cells were counted after 14 ± 2 days.

Transwell migration and Matrigel invasion. Cell motility was assayed using a modified Boyden chamber with 8 μ ethylene terephthalate filters (Becton Dickinson, Lincoln Park, NJ) as described earlier (17). Briefly, a total of 7.5×10^4 cells were incubated in the top chamber of wells in 300 μl serum-free medium containing 0.5% BSA. Cells were allowed to migrate to the lower chamber with standard medium containing 10% FCS for a period of 16 h, fixed in 4% paraformaldehyde, stained with methylene blue and counted. Quadruplicate chambers were used for each cell type and the experiments were repeated 4 times. Recombinant human FGF-2 (1.0 or 10 ng/ml) and/or anti-FGF-2 antibody (50 μg/ml) or β-estradiol (100 ng/ml; Sigma, MO) were added to the upper chamber for the appropriate experiments. The invasive capacities of cells through Matrigel were measured by using similar Boyden chambers pre-coated with 15 μl of reconstituted Matrigel (Becton Dickinson, Lincoln Park, NJ) in standard serum-free medium. A total of 10^5 cells in 300 μl serum-free medium containing 0.5% BSA were placed on top of the Matrigel layer in the upper chamber and allowed to invade through the Matrigel for 24 h to the lower chamber containing standard medium (containing 10% FCS). Invaded cells gathered on the lower side of the membrane were fixed in 4% paraformaldehyde, stained with methylene blue and counted.

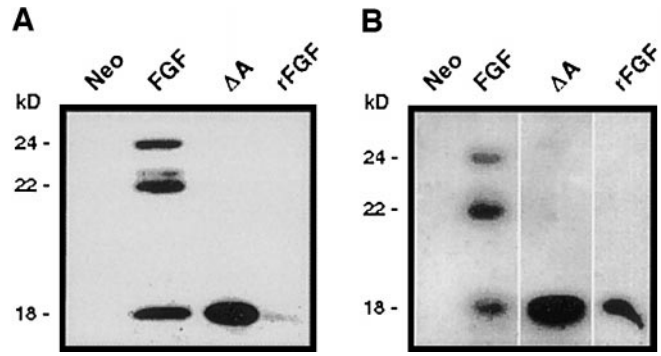

FIG. 1. Western immunoblots of total cell lysates (A) or 2 M NaCl washes (B) from T-47D cells transfected with vector alone (Neo), or vectors coding for full-length FGF-2 (FGF) or 18 kD FGF-2 (ΔA). Recombinant human FGF-2 (rFGF) is loaded as positive control for immunoblots.

Three-dimensional (3D) Matrigel cell culture. Ten thousand cells resuspended in 100 μl of standard culture medium were allowed to spread for 1 h at 37°C in 5% CO_2 on a pre-gelled Matrigel layer made of 150 μl of undiluted Matrigel (Becton Dickinson; Lincoln Park, NJ) in a 24-well tissue culture plate. A second layer of 150 μl of ice-cold Matrigel was overlaid on cells and the incubation was continued for another hour at 37°C in 5% CO_2. After adding 200 μl of culture medium on top of the Matrigel, incubation at 37°C in 5% CO_2 was continued for 2 weeks. Medium added to the top of the Matrigel was replaced every third day. Photographs of the culture were taken after 6 days and 12 days. At the end of the experiment, cells were collected from the Matrigel for biochemical assays using Matrisperse (Becton Dickinson; Lincoln Park, NJ) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Characterization of FGF-2 Expression in T-47D Cells

The expression of various isoforms of FGF-2 was confirmed in selected populations of T-47D cells using standard Western immunoblot techniques (Fig. 1A). Immunofluorescence detection techniques revealed the presence of FGF-2 in both the cytoplasm and the nucleus of T-47D/FGF cells and predominantly in the cytoplasm of T-47D/ΔA cells (data not shown), a pattern reported previously for FGF-2-expressing MDA-MB-231 breast cancer cells (6). FGF-2-expressing cells were also analyzed for their capacity to export FGF-2. Near-confluent cells expressing different isoforms were washed with 2 M NaCl and proteins were concentrated

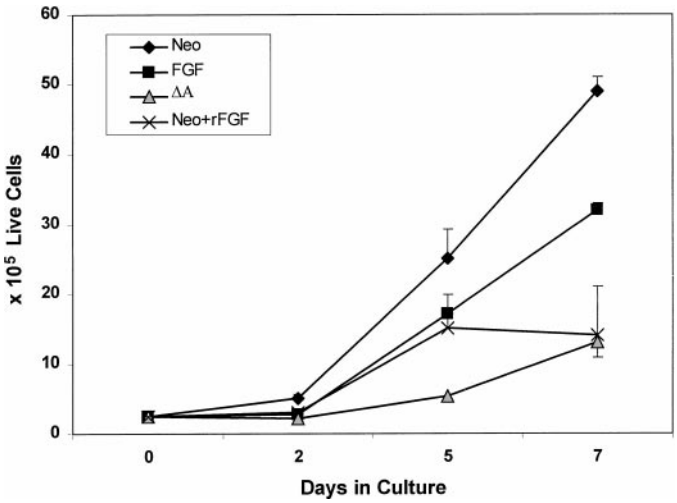

FIG. 2. Effect of FGF-2 on proliferation kinetics of T-47D cells. Cells were seeded (2.5×10^5) in 100-mm plates and incubated at 37°C for 7 days. Triplicate plates were trypsinized at the indicated time points and mixed with 0.4% trypan blue (1:1) and live cells were counted and graphed. Representative data from two independent experiments are shown.

using Centriprep columns (Amicon Inc., Beverly, MA) (6). Western blots of the concentrated 2 M NaCl washes revealed detectable amounts of FGF-2 species that were secreted into the culture medium of cells (Fig. 1B). The exported FGF-2 species corresponded to their intracellular expression patterns. This observation is in agreement with earlier reports that expression of FGF-2 results in their release only in differentiated cell types such as MCF-7, not in poorly differentiated cells such as MDA-MB-231 (6, 19).

Although described as a classical mitogen in a variety of cell types (1, 2, 5), recombinant FGF-2 has been shown to reduce the proliferative capacities of breast cancer cells such as MCF-7 and MDA-MB-134 (16, 20). A similar but diminished growth retarding effect was observed in T-47D/Neo cells when cultured in the presence of 1.0 ng/ml of rhFGF-2 (Fig. 2). Expression of 18 kD FGF-2 (ΔA) also produced a comparable growth inhibitory effect on T-47D cells. Cells expressing all four isoforms of FGF-2 also showed a reduced proliferative response, although marginal, when compared with their vector-transfected controls (Fig. 2). The observed reduction in growth potential of rhFGF-2-treated T-47D cells or FGF-2 expressing derivatives was not due to loss of cell viability as determined by trypan blue assay (data not shown), but most likely due to a prolonged G_1 phase, as reported for MCF-7 cells treated with rhFGF-2 (16). Since FGF-2-expressing T-47D cells also export FGF-2 into the medium, it is possible that the observed inhibitory effect on proliferation could be due to an analogous receptor-mediated inhibitory signaling induced by exported FGF-2 (16).

FGF-2 Expression Inhibits Anchorage-Independent Growth of T-47D Cells

Constitutive expression of FGF-2 confers serum-independent and anchorage-independent growth capabilities to NIH-3T3 fibroblasts (21, 22). On the other hand, enforced expression of FGF-2 reduces the malignant properties in MDA-MB-231 and MCF-7 breast cancer cells (6, 19). We determined whether enforced expression of FGF-2 has a similar effect on T-47D cells that readily form colonies in soft agar (23). Both FGF-2-expressing cell populations showed a significant reduction in their anchorage-independent growth capabilities (Fig. 3). Cells expressing the 18 kD isoform of FGF-2 alone (T47D/ΔA) were found to inhibit the anchorage-independent growth more efficiently than cells expressing all isoforms (T47D/FGF). In addition to the significant reduction in the number of colonies, colonies made up of FGF-2-expressing cells contained significantly fewer numbers of cells than colonies from T-47D/Neo cells. This further suggests that FGF-2 not only impairs the anchorage-independent growth capabilities of T-47D cells but also imposes additional growth restrictions by transducing proliferation-inhibitory signaling.

Inhibition of Matrigel Invasion by Intracellular FGF-2

Although T-47D breast cancer cells present a relatively differentiated phenotype compared to MDA-MB-231 cells, they too have acquired the capacity to invade through Matrigel (24). We tested whether FGF-2 expression has any effect on their invasive capacity by performing overnight Matrigel invasion assays (6). T-47D cells expressing FGF-2 (all isoforms or 18 kD

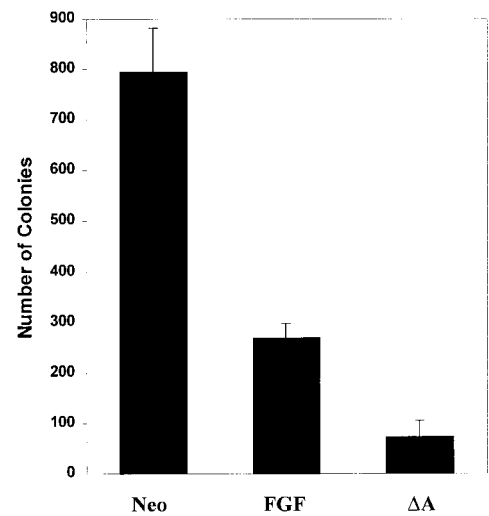

FIG. 3. Colony-forming efficiency of T-47D cells transfected with empty vector (Neo), cells expressing all FGF-2 isoforms (FGF), or 18 kD FGF-2 (ΔA) in 0.3% soft agar. Five thousand cells per dish were incubated for 14 days and colonies of more than 30 cells were counted.

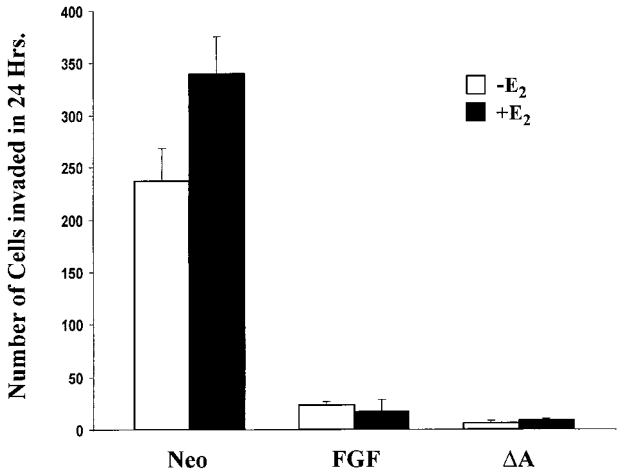

FIG. 4. Invasion of T-47D cells expressing various FGF-2 isoforms in Matrigel-coated modified Boyden chambers with 8-μm pores. Cells were incubated at 5×10^5 cells/chamber with (+E) or without (−E) 100 ng/ml of β-estradiol in the upper chamber. Cells were allowed to invade for 24 h and the filters were stained with methylene blue and counted at 100× magnification.

alone) were significantly inhibited in their invasive potential (Fig. 4). Expression of 18 kD FGF-2 inhibited invasion more strongly than expression all of the isoforms together. This suggested that the expression of the 18 kD isoform is enough to inhibit the invasive phenotype of T-47D cells, even in the presence of HMW isoforms. We reported a similar anti-invasive effect of 18 kD FGF-2 in a more malignant breast cancer cell line MDA-MB-231 (6). In MDA-MB-231 cells, the expression of all isoforms of FGF-2 did not inhibit their invasive potential (6).

Unlike MDA-MB-231 cells, T-47D cells carry wild-type estrogen receptors and respond to estrogen in cell proliferation assays (25). We tested whether the presence of estradiol in the medium had any effect on the invasive behavior of T-47D cells or on the FGF-2-mediated inhibitory effect. In T-47D/Neo cells, analogous to its mitogenic effect (25), estradiol promoted the cells' invasive behavior. However, the pro-invasive effect induced by estradiol was completely blocked by intracellular FGF-2, either alone or in the presence of HMW isoforms (Fig. 4). This further suggests that the anti-invasive effect of FGF-2 is not only inhibitory to the innate motility of T-47D cells, but also blocks the invasion-promoting effect induced by estrogen.

Intracellular FGF-2 Inhibits T-47D Cell Motility

Matrigel invasion is the cumulative effect of a variety of cellular functions, including motility, cellular adhesion and protease activity (26). FGF-2 has been shown to induce expression and activity of a variety of matrix metalloproteinases, including collagenases and caseinases in a variety of cell types including breast cancer cells (6), thereby potentially increasing their capability to invade through basement membrane. However, our previous studies have shown that intracellular 18 kD FGF-2 inhibits invasive behavior of a highly malignant breast cancer cell line MDA-MB-231 by inhibiting their motility (6). To test whether the anti-invasive effect of FGF-2 on relatively more differentiated T-47D cells is mediated through a similar anti-motility effect, we compared the migratory potential of less invasive FGF-2-expressing cells using a standard Boyden chamber technique (17). After 16 h of incubation, cells expressing FGF-2 migrated with markedly reduced efficiency (Fig. 5). The anti-motility effect was more prominent in 18 kD-isoform-expressing cells (Fig. 5), analogous to their invasive behavior (Fig. 4).

Both T-47D derivatives expressing FGF-2 also released their respective FGF-2 isoforms into their culture medium (Fig. 1B), raising the possibility of a receptor-mediated anti-motility signaling. To determine the contribution of the exported FGF-2 to the anti-motility effect of FGF-2 expressed in T-47D cells, we repeated the Boyden chamber experiments after treating the cells with anti-FGF-2 neutralizing antibody and/or exogenous rhFGF-2. Treatment with neutralizing antibody to FGF-2 (50 μg/ml) had no effect on the migratory capacity of either T47D/Neo cells or on the diminished invasive capacity of FGF-2 expressing cells (Fig. 5). Addition of rhFGF-2, on the other hand, increased the motility of T-47D/Neo cells four-fold, an effect that was partially reversed by neutralizing antibody (Fig. 5). Addition of rhFGF-2 had no effect on the

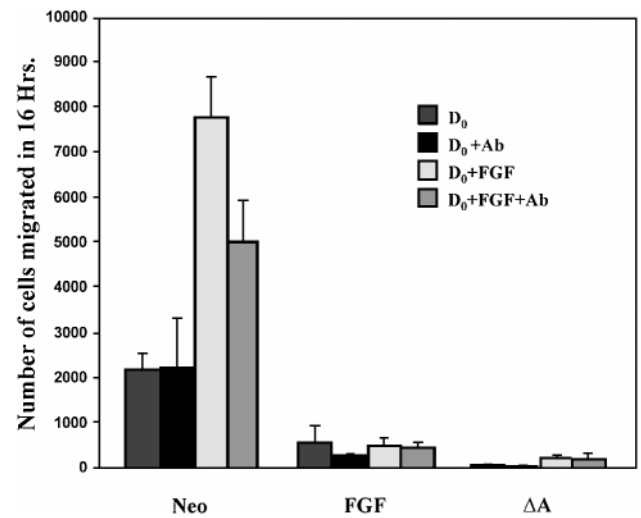

FIG. 5. Migration of T-47D cells expressing various FGF-2 isoforms in a modified Boyden chamber with 8-μm pores. Cells were incubated at 2.5×10^4 cells/chamber without (D_0) or with 50 μg/ml anti-FGF-2 antibody alone (D_0 + Ab), 1 ng/ml rhFGF-2 alone (D_0 + FGF) or both antibody and FGF-2 together (D_0 + FGF + Ab) for 16 h. The filters were stained with methylene blue and counted at 100× magnification.

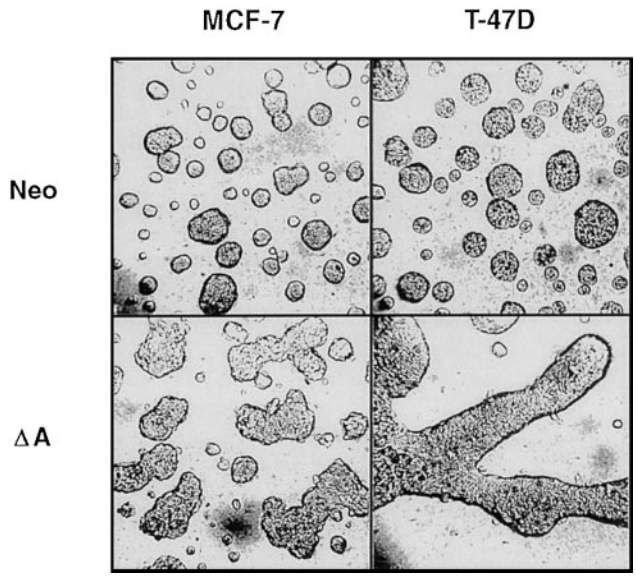

FIG. 6. Photomicrographs (100× magnification) of T-47D and MCF-7 cells transfected with empty vectors (Neo) or expressing 18 kD FGF-2 (ΔA) cultured in 3D Matrigel matrices for 6 days, as described under Materials and Methods.

migratory behavior of either FGF-2-expressing cell type, suggesting that the intracellular expression of FGF-2 is not only inhibitory to migration, but also dominant over the pro-migratory effect of tyrosine kinase receptor-mediated exogenous rhFGF-2 signaling (Fig. 5).

Restricted Migration Promotes Branching Morphogenesis in Breast Cancer Cells

Controlled cell motility is essential for morphogenic differentiation of breast epithelial cells (27, 28). Malignant transformation, on the other hand, promotes aberrant cell motility and abrogates the differentiation process (27). On the basis of the reported *in vivo* observations that strongly suggested an association between FGF-2-expression and a differentiated phenotype in breast cancer, we decided to determine whether the controlled motility observed in FGF-2-expressing cells is associated with the acquisition of the capacity for morphogenic differentiation. We tested this possibility by studying the growth characteristics of FGF-2-expressing T-47D cells in three-dimensional Matrigel culture, a growth condition that approximates the *in vivo* breast epithelial environment (29). We also included MCF-7 cells in this experiment for comparative purposes. MCF-7 is another differentiated breast cancer cell line with demonstrated ability to form branching structures under differentiation promoting condition (30). After 6 days of growth, T-47D cells expressing FGF-2 showed drastic changes in their growth pattern in 3D-Matrigel-cell culture that included prominent tube-like structures with extensive branching (Fig. 6). The branching phenotype was much more pronounced than that of MCF-7/FGF cells that were characterized before as a more differentiated derivative of parental MCF-7 cells. Although appearing to differentiate by morphogenic branching, FGF-2-expressing cells collected from the Matrigel cultures did not produce detectable levels of the milk proteins α-lactalbumin or casein (data not shown). Functional differentiation of breast epithelial cells appears to have two broad physiological outcomes: (a) branching differentiation and (b) alveolar differentiation (31). Although more experiments are needed, a morphological branching phenotype in the absence of production of milk proteins strongly argues for a branching differentiation phenotype for T-47D and MCF-7 cells expressing FGF-2. In summary, our experiments strongly suggest a unique role for FGF-2 in breast epithelial cells in promoting their functional differentiation. Abrogation of this FGF-2 function appears to play a role in allowing breast epithelial cells to undergo the path to dedifferentiation. This argument is further supported by our observation that the three cell types tested, MDA-MB-231 (6), MCF-7 (19) and T-47D, representing different levels of malignant behavior, were all induced to reverse their levels of dedifferentiation by enforced expression of FGF-2. These observations further suggest that potential for the presence of breast epithelial-specific cytoplasmic regulatory molecule(s) that are involved in eliciting a differentiation-promoting effect by FGF-2 in breast epithelial cells. Identifying the presence of such a cell-specific regulatory system will help explain the unique differentiation-promoting role of FGF-2 observed in breast epithelial cells *in vitro* and *in vivo.*

ACKNOWLEDGMENTS

Work was partly supported by the New Jersey State Commission on Cancer Research and the Foundation of the University of Medicine and Dentistry of New Jersey (24-99) (R.K.) and the U.S. Army Breast Cancer Research Program (DAMD17-94-J-4463) (R.W.).

REFERENCES

1. Gospodarowicz, D., Massoglia, S., Cheng, J., Lui, G. M., and Bohlen, P. (1985) Isolation of bovine pituitary fibroblast growth factor purified by fast protein liquid chromatography (FPLC): Partial chemical and biological characterization. *J. Cell. Physiol.* **122,** 323–333.
2. Avivi, A., Zimmer, Y., Yayon, A., Yarden, Y., and Givol, D. (1991) Flg-2, a new member of the family of fibroblast growth factor receptors. *Oncogene* **6,** 1089–1092.
3. Delrieu, I. (2000) The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism. *FEBS Lett.* **468,** 6–10.
4. Lee, P. L., Johnson, E. D., Cousens, L. S., Fried, V. A., and Williams, L. T. (1989) Purification and complementary DNA cloning of a receptor for basic fibroblast growth factor. *Science* **268,** 432–436.

5. Dionne, C. A., Crumley, G., Bellot, F., Kaplow, J. M., Searfoss, G., Ruta, M., Burgess, W. H., Jaye, M., and Schlessinger, J. (1990) Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. *EMBO J.* **9,** 2685–2692.
6. Korah, R. M., Sysounthone, V., Golowa, Y., and Wieder, R. (2000) Basic fibroblast factor confers a less malignant phenotype in MDA-MB-231 human breast cancer cells. *Cancer Res.* **60,** 733–740.
7. Klagsbrun, M., Smith, S., Sullivan, R., Shing, Y., Davidson, S., Smith, J. A., and Sasse, J. (1987) Multiple forms of basic fibroblast growth factor: Amino-terminal cleavages by rumor cell- and brain cell-derived acidic proteinase. *Proc. Natl. Acad. Sci. USA* **84,** 1839–1843.
8. Presta, M., Statuto, M., Rusnati, M., Dell'Era, P., and Ragnotii, G. (1989) Characterization of a M_r 25,000 basic fibroblast growth factor form in the adult, regenerating and fetal rat liver. *Biochem. Biophys. Res. Commun.* **164,** 1182–1189.
9. Arnaud, E., Touriol, C., Boutonnet, C., Gensac, M.-C., Vagner, S., Prats, H., and Prats, A.-C. (1999) A new 34-kilodalton isoform of human fibroblast growth factor 2 is Cap dependently synthesized by using a non-AUG start codon and behaves as a survival factor. *Mol. Cell. Biol.* **19,** 505–514.
10. Bikfalvi, A., Klein, S., Pintucci, G., Quarto, N., Mignatti, P., and Rifkin, D. B. (1995) Differential modulation of cell phenotype by different molecular weight forms of basic fibroblast growth factor: Possible intracellular signalling by the high molecular weight forms. *J. Cell. Biol.* **129,** 233–243.
11. Quarto, N., Finger, F. P., and Rifkin, D. B. (1991) The NH2-terminal extension of high molecular weight bFGF is a nuclear targeting signal. *J. Cell. Physiol.* **147,** 311–318.
12. Florkiewicz, R. Z., and Sommer, A. (1989) Human basic fibroblast growth factor gene encodes four polypeptides: Three initiate translation from non-AUG codons. *Proc. Natl. Acad. Sci. USA* **86,** 3978–3981.
13. Couderc, B., Prats, H., Bayard, F., and Amalric, F. (1991) Potential oncogenic effects of basic fibroblast growth factor requires cooperation between CUG and AUG-initiated forms. *Cell Regul.* **2,** 709–718.
14. Laqmani, Y., Graham, M., and Coombes, R. C. (1992) Expression of basic fibroblast growth factor, FGFR1 and FGFR2 in normal and malignant human breast, and comparison with other normal tissue. *Br. J. Cancer* **66,** 772–776.
15. Gomm, J. J., Smith, J., Ryall, G. K., Baille, R., Turnbull, L., and Coombes, R. C. (1991) Localization of basic fibroblast growth factor and transforming growth factor-beta1 in the human mammary gland. *Cancer Res.* **51,** 4685–4692.
16. Wang, H., Rubin, M., Fenig, E., DeBlasio, T., Mendelsohn, J., Yahalom, J., and Wieder, R. (1997) Basic FGF causes growth arrest in MCF-7 human breast cancer cells while inducing both mitogenic and inhibitory G_1 events. *Cancer Res.* **57,** 1750–1757.
17. Yu, D., Qu, C., Henegariu, O., Lu, X., and Feng, G. (1998) Protein-tyrosinephosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. *J. Biol. Chem.* **273,** 225–21131.
18. Berdichevsky, F., Alford, D., D'Souza, B., and Taylor-Papadimitriou, U. (1994) Branching morphogenesis of human mammary epithelial cells in collagen gels. *J. Cell Sci.* **107,** 3557–3568.
19. Wieder, R., Fenig, E., Wang, H., Wang, Q., Paglin, S., Menzel, T., Gabrilove, J., Fuks, Z., and Yahalom, J. (1998) Overexpression of basic fibroblast growth factor in MCF-7 human breast cancer cells: Lack of correlation between inhibition of cell growth and MAP kinase activation. *J. Cell. Physiol.* **177,** 411–425.
20. McLeskey, S. W., Ding, I. Y., Lippman, M. E., and Kern, F. G. (1994) MDA-MB-134 breast carcinoma cells overexpress fibroblast growth factor (FGF) receptors and are growth-inhibited by FGF ligands. *Cancer Res.* **54,** 523–530.
21. Renko, M., Quarto, N., Morimoto, T., and Rifkin, D. B. (1991) Nuclear and cytoplasmic localization of different basic fibroblast growth factor species. *J. Cell. Physiol.* **109,** 1–4.
22. Wieder, R., Wang, H., Shirke, S., Wang, Q., Menzel, T., Feirt, N., Jakubowski, A. A., and Gabrilove, J. L. (1997) Low level expression of basic FGF upregulates Bcl-2 and delays apoptosis, but high intracellular levels are required to induce transformation in NIH 3T3 cells. *Growth Factors* **15,** 41–60.
23. Tseng, M. T., and Safa, A. R. (1983) Light, fluorescent, and electron microscopic analysis of cultured breast tumor cells (T-47D) treated with 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] dihydrochloride. *Cancer Res.* **43,** 5910–5914.
24. Yee, C., and Shiu, R. P. C. (1986) Degradation of endothelial basement membrane by human breast cancer cell lines. *Cancer Res.* **46,** 1835–1839.
25. Fasco, M. J. (1997) Quantitation of estrogen receptor mRNA and its alternatively spliced mRNAs in breast tumor cells and tissues. *Anal. Biochem.* **245,** 167–178.
26. van den Brule, F., Engel, J., Stetler-Stevenson, W. G., Liu, F., Sobel, M. E., and Castronovo, V. (1992) Genes involved in tumor invasion and metastasis are differentially modulated by estradiol and progestin in human breast-cancer cells. *Int. J. Cancer* **52,** 653–657.
27. Peterson, O. W., Ronnov-Jessen, L., Weaver, V. M., and Bissell, M. J. (1998) Differentiation and cancer in the mammary gland: Shedding light on the old dichotomy. *Adv. Cancer Res.* **98,** 135–161.
28. Quaranta, V. (2000) Cell migration through extracellular matrix: Membrane-type metalloproteinases make the way. *J. Cell Biol.* **149,** 1167–1169.
29. Streuli, C. H., Bailey, N., and Bissell, M. J. (1991) Control of mammary epithelial differentiation: Basement membrane induces tissue-specific gene expression in the absence of cell–cell interaction and morphological polarity. *J. Cell Biol.* **115,** 1383–1395.
30. Lalani, E. N., Williams, R., Jayaram, Y., Gilbert, C., Chaudhary, S., Siu, L., Koumarianou, A., Playford, R., and Stamp, G. W. H. (1999) Trefoil factor-2, human spasmolytic polypeptide, promotes branching morphogenesis in MCF-7 cells. *Lab. Invest.* **79,** 537–546.
31. Niemann, C., Brinkmann, V., Spitzer, E., Hartmann, G., Sachs, M., Naundorf, H., and Birchmeier, W. (1998) Reconstitution of mammary gland development *in vitro:* Requirement of c-met and c-erbB2 signaling for branching and alveolar morphogenesis. *J. Cell Biol.* **143,** 533–545.