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Transforming Growth Factor-β3 Increases Gap-Junctional Communication among Folliculostellate Cells to Release Basic Fibroblast Growth Factor

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Abstract

Folliculostellate (FS) cells are known to communicate with each other and with endocrine cells via gap junctions in the anterior pituitary. We investigated whether TGFβ3 and estradiol, known to regulate FS cell production and secretion of basic fibroblast growth factor (bFGF), increases gap junctional communication to alter bFGF secretion from FS cells. FS cells in monolayer cultures were treated with TGFβ3 or vehicle alone for 24 h and then microinjected with Lucifer Yellow and high-molecular-weight Texas Red dextran. Ten minutes later the transfer of dye among adjacent cells was recorded with a digital microscope. TGFβ3 increased the transfer of dye. The TGFβ3-neutralizing antibody and the gap junction inhibitor octanol reduced the effect of TGFβ3 on the transfer of dye. The TGFβ3-induced transfer of dye was unaltered by simultaneous treatment with estradiol. The steroid alone also had no effect. TGFβ3 increased total and phosphorylated levels of connexin 43. Estradiol treatment did not produce any significant changes on basal or TGFβ3-induced increases in connexin 43 levels. The gap-junction inhibitor octanol reduced TGFβ3-increased levels of bFGF in FS cells. Taken together, these results suggest that TGFβ3 may act on FS cells to increase gap-junctional communication to maximize its effect on bFGF secretion.

Folliculostellate (FS) cells of the anterior pituitary are S-100-positive cells thought to be important for the regulation of pituitary endocrine cell functions (1,2). FS cells have long and complex processes that interact with each other forming a mesh around endocrine cells (1). They also secrete factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor, and IL-6 (3). Recently it has been suggested that gap-junctional communication may be important for the function of FS cells (4,5).

Gap junctions are composed of transmembrane channels joining the interiors of adjacent cells (6–11). These junctions allow cell-to-cell exchange of cytoplasmic molecules like ions and small polar molecules including water, amino acids, calcium, inositol 1,4,5,-triphosphate (IP3), cAMP, sugars, and small peptides of 1000–1200 Da (12–17). A large body of evidence suggests that gap junctions are involved in controlling growth and differentiation by allowing cell-to-cell transfer of signal molecules and metabolites (18–20). The exchange through gap junctions between the neighboring cells is dependent on the functional state of the channels, which can be estimated by measuring either the electrical coupling (*e.g.* current transfer or gap-junctional conductance) (21) or the dye coupling (*e.g.* transfer of a fluorescent probe from an injected cell to its neighboring cells) (22). The effects

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of TGFβ3 on gap-junctional communication in the pituitary cells have not been studied. However, TGFβ1 and forskolin modulate gap-junctional communication of Schwann cells, close cellular phenotypes of FS cells because both cell types produce the immunoreactive S-100 protein (23).

The TGFβ family of peptides has been shown to be involved in the regulation of proliferation and secretion of various pituitary cells including lactotropes (24,25). TGFβ3 in particular, secreted by lactotropes, has been shown to increase the function of FS cells (2). FS cells, in turn, secreted bFGF and caused the proliferation of lactotropes (2). This interaction among FS cells and lactotropes seems to have relevance to prolactinomas, tumors of the prolactin-secreting lactotropes in the pituitary gland, especially in an estrogen-rich environment (24). Recent studies have identified the regulatory role of TGFβ-related peptides in estradiol's action on lactotropes (24,25). Moreover, it was shown that TGFβ3 and bFGF interact to facilitate the communication among lactotropes and FS cells, which is necessary for the mitogenic action of estradiol (24).

Gap junctions connect the cytoplasmic domains of contacting cells to allow metabolic and ionic exchange between them (26). Gap junctions consist of channel proteins called connexins, a family of proteins that, upon phosphorylation, increase permeabilization of the cells to molecules, which are permeable through gap junctions (26). There are various types of connexins. Of these, connexin 43 (Cx43) is known to express in astrocytes and in the pituitary (4,27–29). It was shown that Cx43 is present in the mink pituitary and that this protein's level changes with the seasons of the year and during pregnancy (3,4). It has been suggested that Cx43 is found in the FS cell based on its distribution pattern in the pituitary (4). However, the production of Cx43 and the effect of TGFβ3 on this protein in the presence and absence of estradiol in rat FS cells have not been determined. Therefore, we tested whether, in either the presence or absence of estradiol, TGFβ3 increases Cx43 expression and gap-junctional communication in FS cells. Here we report that TGFβ3, but not estradiol, increased Cx43 activation and gap-junctional communication to increase the release of bFGF from FS cells.

Materials and Methods

Folliculostellate cell cultures

An FS cell line established from anterior pituitary cells from cyclic female Fischer-344 rats was used (30). The cell line was maintained in DMEM/F-12 media with heat-inactivated 10% fetal bovine serum. Before gap-junction study, 1×10^6 cells/well were maintained for 2 d in DMEM/F-12 medium containing serum supplement (consisting of 100 μM human transferrin, 5 μM insulin, 1 μM putrescine, and 30 nM sodium selenite) in 6-well plastic dishes. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless noted otherwise.

Dye transfer study

FS cultures were treated with various concentrations of TGFβ3 prepared in fresh DMEM/F12 medium containing serum supplement at the indicated doses or with vehicle (4 mM HCL and 0.1% BSA) for 24 h before microinjection of the dye. TGFβ3 and the TGFβ3 blocking antibody were obtained from R&D Systems, Inc. (Minneapolis, MN). The blocking antibody was used at a concentration of 10 μg/ml with or without 10 ng/ml of TGFβ3 for a period of 24 h before microinjection. Antirabbit γ-globulin (ARGG; Calbiochem, San Diego, CA) was used at a concentration of 10 μg/ml. In some cultures octanol, known to block gap-junctional communication (31,32), was used. The blocker was used at concentrations of 1 mM and added to the culture 30 min before micro-injection of the dye.

Confluent cultures were microinjected with Lucifer Yellow and a high-molecular-weight dextran (molecular weight 10,000 or 70,000) coupled to Texas Red (Molecular Probes, Eugene, OR). Care was taken to inject the dye in only one cell. We used an Eppendorf Injectman N12 microinjection system and a vibration-isolated platform. The amount of dye transfer to other cells was recorded 10 min after injection. Phase contrast, fluorescein isothiocyanate channel, and Texas Red channel images were photographed separately with appropriate filters.

Dye transfer was recorded with a charge-coupled device camera coupled to a TE-2000 inverted phase-contrast microscope equipped with epi-fluorescence (Nikon, Tokyo, Japan). Phase and double-fluorescent digital images in the fluorescein isothiocyanate and Texas Red channels were merged using Photoshop (version 7; Adobe, San Jose, CA). The number of Lucifer Yellow-positive cells (*green* in the figures) was counted visually in the growth factor-treated and control cultures.

Immunostaining for Cx43

FS cells (10,000 cells/well) were grown on 8-chamber slide plates in serum-containing medium. After 2 d of plating, the medium was changed to serum-free, chemically defined medium. On the following day, cells were treated for 24 h with vehicle (control) or TGFβ3 (10 ng/ml) in serum supplement media. Cultures were washed with 0.1 M PBS for 5 min, fixed with 4% formaldehyde for 15 min, and then washed in 0.05 M Tris-HCl buffer (pH 7.6). These cultures were blocked for 1 h with Tris-HCl buffer containing 10% horse serum, 0.2% Triton X-100, and 0.1% sodium azide. Cultures were treated overnight at 4 C with primary rabbit anti-Cx43 (1:1000; Zymed Laboratory Inc., San Francisco, CA) in the blocking buffer. They were washed in Tris-HCl buffer and then incubated for 1 h with goat biotinylated antirabbit IgG (H+L) secondary antibody (1:3000; Vector Laboratories Inc., Burlingame, CA) in the blocking buffer. Cell-containing chambers were washed with Tris-HCl, dried, and mounted using 4′,6′-diamino-2-phenylindole-containing mounting medium (H-1200; Vector Laboratories).

Western blot for Cx43

FS cells (500,000/well) were grown in 6-well plates in serum-containing medium. After 2 d of plating, the medium was changed to serum-free medium. On the following day, cells were treated for 3 or 24 h with vehicle (control), TGFβ3 (10 ng/ml), estradiol (10 nM), or octanol (1.0 mM) in media containing serum supplement. Cells were then lysed in sample gel loading buffer containing Tris-HCl (pH 6.8), 2% glycerol, 0.05% bromophenol blue, 10% glycerol, and 5% β-mercaptoethanol (14.4 M) and heated at 95 C for 5–6 min. Each sample was resolved on SDS-PAGE and transferred to Immobilon-P polyvinyl difluoride membranes (Millipore, Bedford, MA). Membranes were incubated for 1 h with anti-Cx43 antibody or anti-actin antibody (BD Biosciences, Palo Alto, CA) at room temperature in 5% milk, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20. Membranes were then washed, incubated for 1 h with peroxidase-conjugated antimouse antibody, and developed using enhanced chemiluminescence reagent (Amersham, Piscataway, NJ). For quantification of Cx43 levels, the band intensities of Cx43 or phosphorylated Cx43 were determined using Scion Image software (Scion Corp., Frederick, MD) and normalized to the corresponding actin band intensities.

bFGF assay

FS cells (500,000/well) were grown in 6-well plates in serum-containing medium. After 2 d of plating, the medium was changed to serum-free medium. On the following day, cells were treated for 3 h with vehicle (control), TGFβ3 (10 ng/ml), estradiol (10 nM), or octanol (1.0 mM) in medium containing serum supplement media. Cell supernatants were collected and

assessed for bFGF levels using Quantikine immunoassay kits (R&D Systems). Cells were lysed in 100 μl of lysis buffer containing 150 mM Tris-HCl (pH 7.5), 300 mM NaCl, 0.1% Triton X-100, and a protease inhibitor cocktail (Sigma). Total protein concentration in each cell lysate was determined using the Bio-Rad assay to normalize the bFGF release per milligram of total protein.

Statistical analysis

The data shown in the figures and the text are means ± SEM. Comparisons between two groups were made using *t* tests. Data comparisons among multiple groups were done using one-way ANOVA. *Post hoc* tests involved the Student-Newmann-Keuls test. A value of $P < 0.05$ was considered significant.

Results

Effect of TGF β3 and estradiol on FS cell gap-junctional communication

To determine whether TGFβ3 modulates gap-junctional communication, FS cells were treated for 24 h with or without various doses of TGFβ3. Afterward, cells were microinjected with the low-molecular-weight, gap-junction passing, fluorescent probe Lucifer Yellow, and the high-molecular-weight, gap-junction nonpassing, dextran coupled to a fluorescent probe, Texas Red (10 kDa). Figure 1 shows the dose-related effect of TGFβ3. After 10 min of microinjection in control cultures treated with vehicle alone, Lucifer Yellow spread to 24.5 ± 3.5 cells (n = 46). After TGFβ3 treatment with 5 ng/ml for 24 h, low-molecular-weight dye, but not the high-molecular-weight dextran-coupled fluorescent probe, spread was seen in approximately 2-fold more neighboring cells. This value was significantly higher ($P < 0.01$) than the number in the control group. Treatment with 10 ng/ml TGFβ3 showed Lucifer Yellow dye spread in approximately 6-fold more ($P < 0.001$) neighboring cells. Compared with 5 ng/ml TGFβ3, the 10 ng/ml TGFβ3 treatment caused a significantly higher dye spread ($P < 0.001$), indicating a dose-dependent response to TGFβ3 treatment. Interestingly, when cells were treated for 24 h with 10 ng/ml TGFβ3 simultaneously with a TGFβ3-neutralizing antibody (10 μg/ml), the Lucifer Yellow dye spread to neighboring cells was similar to the control values. In a preliminary study in which a small number of cultures (n = 3) were treated with the neutralizing antibody alone, dye transfer was seen in the number of cells (22 ± 7) similar to those in the control group (Fig. 1), suggesting that the antibody by itself had no effect on dye transfer. When cells were treated with TGFβ3 and the nonspecific immunoglobulin ARGG, dye transfer occurred in the same number of cells as in the group treated with growth factor alone, suggesting that the increase in dye transfer was specifically due to TGFβ3 and not due to the nonspecific presence of protein or antibodies.

To determine whether the TGFβ3-induced dye transfer was due to increased gap-junctional functionality, we incubated growth-factor-treated cells with octanol, which is known to block gap-junctional communication (31,32). The number of cells with dye after octanol (14 ± 2; n = 6; Fig. 2, A and B) was lower ($P < 0.05$) than the control (24.5 ± 3.5; n = 46; Fig. 1, A and B). Octanol reduced ($P < 0.001$) the dye transfer in TGFβ3-treated cultures (Fig. 2, A–F), suggesting that a significant portion of the dye transfer in TGFβ3-treated cultures was due to gap-junctional communication.

Because estradiol has been shown to increase FS cell activity (24,33), we surmised that estrogen by itself or in the presence of TGFβ3 would enhance dye transfer. However, as shown in Fig. 3, cells treated with estradiol showed dye transfer similar to controls (number of cells with dye; control, 24.5 ± 3.5; n = 46; estradiol, 17.0 ± 5.8; n = 12; Figs. 1, A and B, and 3, C and D). In the presence of TGFβ3 (10 ng/ml), estradiol did not increase dye spread,

compared with the same amount of TGFβ3 alone, suggesting that TGFβ3 action is independent of estradiol.

Effect of TGFβ3 and estradiol on gap-junctional protein Cx43 expression in FS cells

Gap-junctional communication between cells is regulated by the opening of endogenous hemichannels upon phosphorylation of various connexin proteins (34,35). Of these proteins, Cx43 has been shown to be expressed in the pituitary (36) and among FS cells (4,37). Hence, we determined whether Cx43 is expressed in FS cells in culture and regulated by TGFβ3 and/or estradiol.

Fluorescence microscopy studies of Cx43 immunoreactivity in FS cell cultures, using a commercially available specific antibody for Cx43, identified many FS cells stained with Cx43 (Fig. 4). The number of Cx43-positive cells was significantly increased ($P < 0.01$) after 10 ng/ml TGFβ3 treatment. Visual comparisons of the cultures treated with TGFβ3 and with control suggest that the intensity of the Cx43 staining per cells was increased by TGFβ3 (Fig. 4). Estradiol-17 β (10 nM) treatment failed to significantly affect the number of Cx43 immunoreactive FS cells (data not shown).

The TGFβ3-induced alteration of Cx43 levels in FS cells was measured by Western blot. Because Cx-43 phosphorylation is known to open the hemichannels and gap junctions, the phosphorylated and nonphosphorylated protein levels of Cx43 in FS cells were determined. As shown in Fig. 5, the treatment of FS cells with the dose of TGFβ3 (10 ng/ml) significantly ($P < 0.001$) elevated phosphorylated, nonphosphorylated, and total levels of Cx43. As in the case of dye transfer, Western blot data did not reveal any stimulatory action of estradiol on the cellular level of phosphorylated or nonphosphorylated connexin proteins.

Effect of a gap-junctional blocker on TGFβ3-induced bFGF release from FS cells after 3 h treatment

To determine whether a TGFβ3-induced increase in gap-junctional communication affects the secretory function of FS cells, we determined the effects TGFβ3 on bFGF secretion and levels of Cx43 in FS cells after 3 h of treatment. We found that TGFβ3 (10 ng/ml) significantly increased the bFGF release and phosphorylation of Cx43 in FS cells after 3 h of treatment (Fig. 6, A and B). Furthermore, the dose of octanol (1 mM) that significantly affected dye transfer in FS cells also significantly reduced the TGFβ3-induced increase in bFGF release from these cells. The blocker did not affect the basal release of bFGF. These results suggest that gap-junctional communication may be important to maximize TGFβ3's effect on bFGF release.

Discussion

In this paper we have shown that, in FS cells, TGFβ3 caused a dose-dependent increase in Lucifer Yellow dye transfer between the cells that was blocked by the TGFβ3 function-blocking antibody and by the gap-junctional blocker octanol. The enhanced dye transfer by TGFβ3 was associated with an increased immunoreactive Cx43 levels and phosphorylated Cx43 content in FS cells. TGFβ3 treatment for 3 h increased bFGF release from FS cells that was associated with increased phosphorylation of Cx43 in these cells. Additionally, octanol reduced TGFβ3-induced bFGF release from FS cells. Estradiol failed to increase dye transfers and Cx43 phosphorylation in FS cells. This is the first report that TGFβ3 causes increased gap-junctional communication between FS cells to maximize its effect on bFGF release from these cells.

In the rat pituitary, formation of gap junctions between FS cells has been demonstrated using electron microscopy methods (38,39). It has also been shown that FS cells communicate

with other FS cells and endocrine cells, including prolactin-secreting lactotropes (5). Data obtained from our present study are in agreement with the observation that FS cells have functional gap junctions among them. One of the structural proteins of a gap junction, Cx43, has also been shown to express between FS cells in the mink anterior pituitary (4,37). Detection of gap-junctional protein Cx43 in FS cells and the evidence of dye transfer between FS cells suggest that gap-junctional communication exists among FS cells.

The gap-junctional number in the anterior pituitary varied during the estrous cycle; it was highest in proestrous and estrous and increased during middle to late pregnancy in rats (38). This suggests that the gonadal factors such as estradiol may regulate the rate of occurrence of gap-junction in the anterior pituitary gland. Our data, which show no changes in dye transfer or Cx43 after estradiol, indicate that the steroid had no direct effect on the rate of occurrence and molecular transfer across gap junctions in the FS cells. The difference between these two observations could be explained by the fact that progesterone, which is elevated during the proestrous and estrous phases and during pregnancy, enhances the rate of gap junction formation between FS cells in the pituitary (39). Also, estradiol has been shown to increase production of TGFβ3 from the lactotropes in the pituitary (2), and TGFβ3 increases Cx43 levels and dye transfer in FS cells. Hence, estradiol's gap-junctional communication-enhancing action in the *in situ* pituitary might be indirectly via increasing TGFβ3 production from the lactotropes.

In the mink anterior pituitary, expression of Cx43 in FS cells was elevated during the spring and during lactation when the lactotrope cell number and prolactin secretion rate were very high (4). During these reproductive phases, enhanced expression of Cx43 in FS cells could have resulted from elevated TGFβ3 production and secretion due to increased lactotropic cell number. In this study we found that, in FS cells, TGFβ3 increased dye transfer, Cx43 phosphorylation and bFGF release, and all of these actions were suppressed by the gap-junctional blocker octanol. Together these data support a physiological role for TGFβ3 in increasing gap-junctional communication to maximize its effect on FS cells.

Within the anterior pituitary, FS cells are known to form a cell network, which is involved in the propagation of cytosolic calcium waves via gap junctions after electrical stimulation (40). Furthermore, in the rat pituitary, these cells are believed to be involved in synchronization of intrapituitary electrical and calcium signaling (1,41). Injection of IP3 into FS cells has been shown to initiate intercellular calcium waves that can be blocked by gap-junctional channel blockers (40). In this context, it is interesting to note that TGFβ alters calcium influx in fibroblasts (42) and that TGFβ-induced calcium influx involves the IP3 receptor (43). It could be hypothesized that the observed TGFβ3-increased gap-junctional communication in FS cells plays a role in the coordination of the propagation of cytosolic calcium waves between these cells. However, this role of TGFβ-related peptides needs to be experimentally verified by determining the effect of the growth factor on cytosolic calcium waves between the cells.

Because FS cells were shown to be activated by the TGFβ3 secreted by the lactotropes (2), increased gap-junctional communication after TGFβ3, which is reported in this paper, may also underlie the modulatory role of FS cells in pituitary function and pathology. For instance, increased gap-junctional communication could lead to more FS cells being activated by lactotropes that could then lead to more bFGF release by the FS cells that, in turn, could increase more lactotropic cell growth (Fig. 7). In conclusion, the finding of this paper may have relevance to TGFβ3's action on FS cells in terms of its physiological function, including bFGF release and calcium influx or pathological development, such as lactotropic cell tumors.

Acknowledgments

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Abbreviations

ARGG	Antirabbit γ-globulin
bFGF	basic fibroblast growth factor
Cx43	connexin 43
FS	folliculostellate
IP3	inositol 1,4,5,-triphosphate

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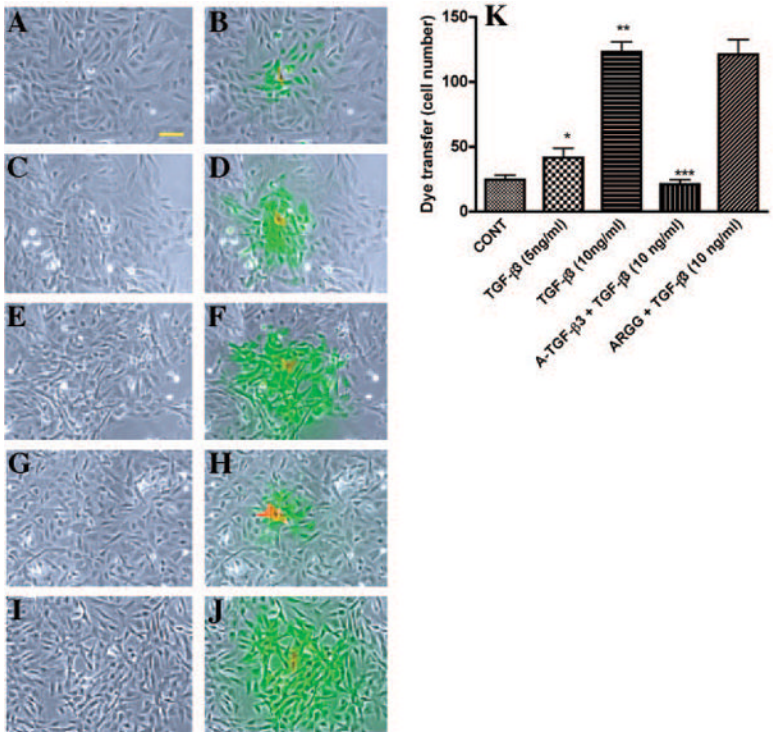

Fig. 1.

Effect of TGFβ3 on dye transfer in FS cells. Confluent cultures of FS cells were treated for 24 h with either vehicle (CONT; A and B), 5 ng/ml TGFβ3 (C and D), 10 ng/ml TGFβ3 (E and F), 10 μg TGFβ3 neutralizing antibody (A-TGFβ3) with 10 ng/ml of TGFβ3 (G and H), or 10 μg ARGG with 10 ng/ml of TGFβ3 (I and J). One cell in each culture was microinjected with Lucifer Yellow and 10 kDa Texas Red dextran. Representative phase-contrast (A, C, E, G, and I) and phase overlaid with double-channel fluorescence (B, D, F, H, and J) images taken 10 min after dye injection are shown: Lucifer Yellow in *green* and Texas Red dextran in *red*. Overlapping regions appear as *dark yellow*. *Scale bar*, 10 μM. The number of Lucifer Yellow-positive cells counted 10 min after injection was used as the measure of dye transfer. Mean ± SEM values of Lucifer Yellow-positive cells are shown in the histogram (K); n = 9–46. *, $P < 0.001$, significantly different from the CONT-treated group; **, $P < 0.001$, significantly different from the CONT and the 5 ng/ml TGFβ3-treated groups; ***, $P < 0.001$, significantly different from the 10 ng/ml of TGFβ3-treated groups.

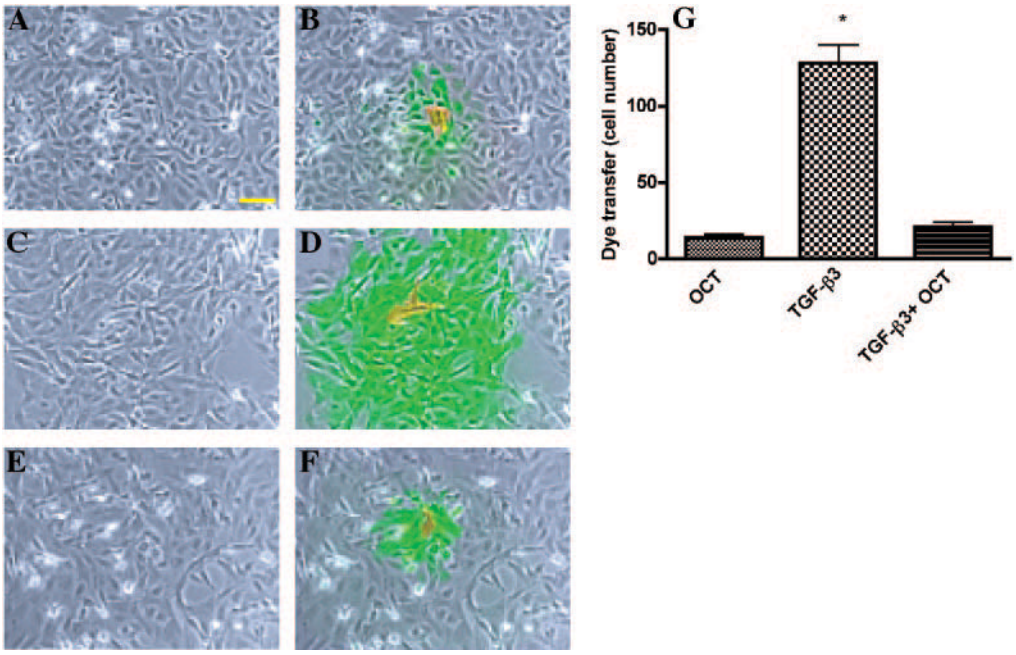

Fig. 2.

Influence of octanol on TGFβ3-induced dye transfer in FS cells. Representative phase-contrast (A, C, and E) and phase overlaid with double-channel fluorescence (B, D, and F) images of cultures treated with 1 mM octanol (OCT; A and B), 10 ng/ml TGFβ3 (C and D), or 10 ng/ml TGFβ3 with 1 mM octanol (TGFβ3 + OCT; E and F) are shown: Lucifer Yellow is shown in *green* and Texas Red dextran in *red*. *Scale bar*, 10 μM. Mean ± SEM values of Lucifer Yellow-positive cells are shown in the histogram (G); n = 9–12. *, $P < 0.001$, significantly different from the TGFβ3 + OCT-treated group.

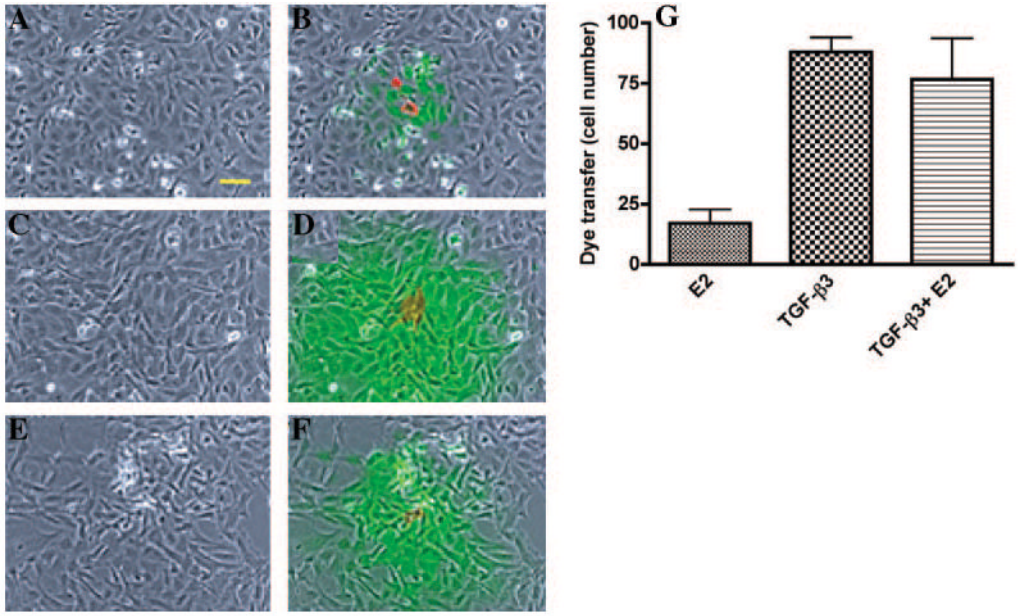

Fig. 3.

Influence of estradiol on TGFβ3-induced dye transfer in FS cells. Representative images of phase-contrast (A, C, and E) and phase overlaid with double-channel fluorescence (B, D, and F) cultures treated with 10 nM estradiol-17β (E2; A and B), 10 ng/ml TGFβ3 (TGFβ3; C and D), or 10 nM E2 with 10 ng/ml TGFβ3 (TGFβ3 + E2; E and F) are shown: Lucifer Yellow in *green* and Texas Red dextran in *red. Scale bar*, 10 μM. Mean ± SEM values of Lucifer Yellow-positive cells are shown in the histogram (G); n = 9–12.

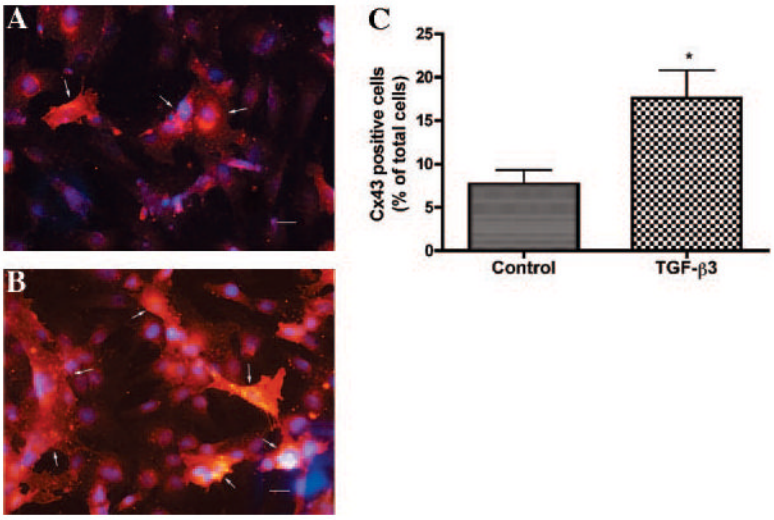

Fig. 4.

Effects of TGFβ3 on Cx43 immunoreactivity in FS cells. Representative microphotographs showing Cx43 immunoreactivity in FS cells treated for a period of 24 h with vehicle (A) or TGFβ3 (10 ng/ml; B). *Arrows* identify some of the Cx43 immunopositive cells. −, 10 μm. C, Means ± SEM values of the percentage of the total cells that were Cx43 immunoreactive are shown; n = 3. *, $P < 0.05$, compared with control (CONT).

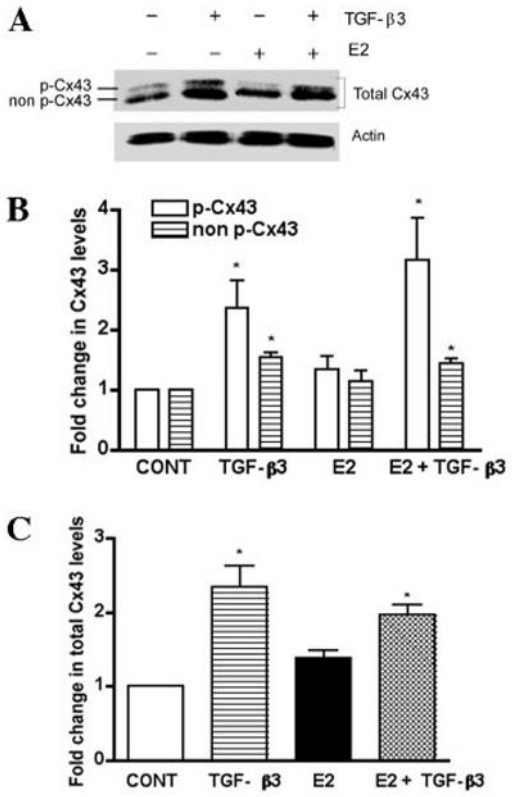

Fig. 5.

Effects of TGFβ3 and estradiol on phosphorylation and total Cx43 levels in FS cells. FS cells were treated for 24 h with TGFβ3 (10 ng/ml) and estradiol(E2; 10 nM) alone or in combination. The levels of Cx43 were determined using an anti-Cx43 antibody. The antibody detects nonphosphorylated and phosphorylated forms of Cx43; a high-molecular-size band represents phosphorylated Cx43 (p-Cx43) and lower-size band represents nonphosphorylated Cx43. Total protein includes both the bands. A, Representative Western blot. B, Densitometric analysis showing changes in levels of p-Cx43 and non-p-Cx43 levels. C, Densitometric analysis showing changes in levels of total Cx43. *Each bar* represents mean ± SEM of three to five individual experiments. *, $P < 0.05$, compared with the respective control group.

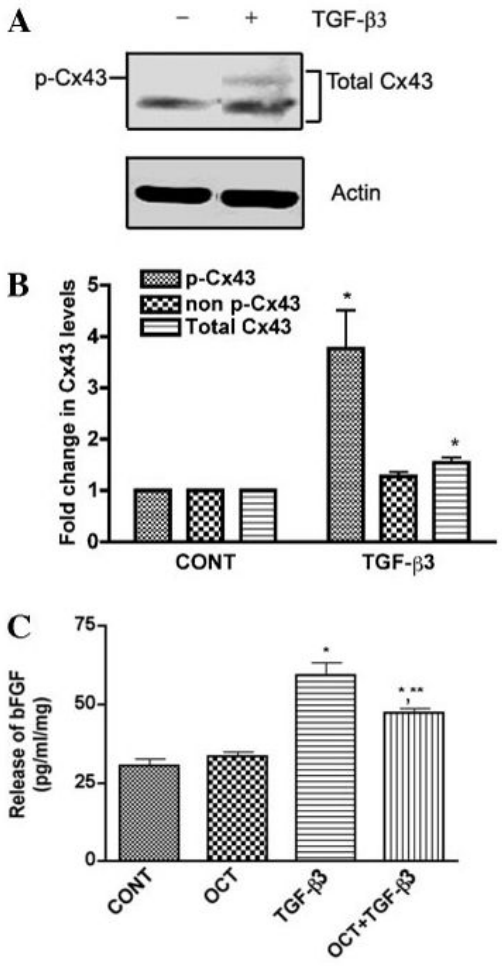

Fig. 6.

Role of gap-junctional communication in TGF*β*3-induced bFGF release from FS cells. The effects of TGF*β*3 (10 ng/ml) treatment for a period of 3 h was determined on phosphorylated (p-Cx43) or nonphosphorylated Cx43 proteins levels (A and B) and bFGF release in the presence and absence of octanol (OCT; C) from FS cells. A, Representative Western blots showing the effect of TGF*β*3 on p-Cx43, non-p-Cx43, and total Cx43 levels. B, Densitometric analysis showing changes in p-Cx43, non-p-Cx43, and total Cx43 levels after TGF*β*3 treatment. Each *bar* represents mean ± SEM of four individual experiments. *, $P < 0.05$, compared with respective control (CONT) groups. C, Effect of octanol on TGF*β*3-induced increase on bFGF release from FS cells. The level of bFGF in the culture media was assessed using immunoassay. Each *bar* represents mean ± SEM of four individual experiments. *, $P < 0.05$, compared with the respective control group.

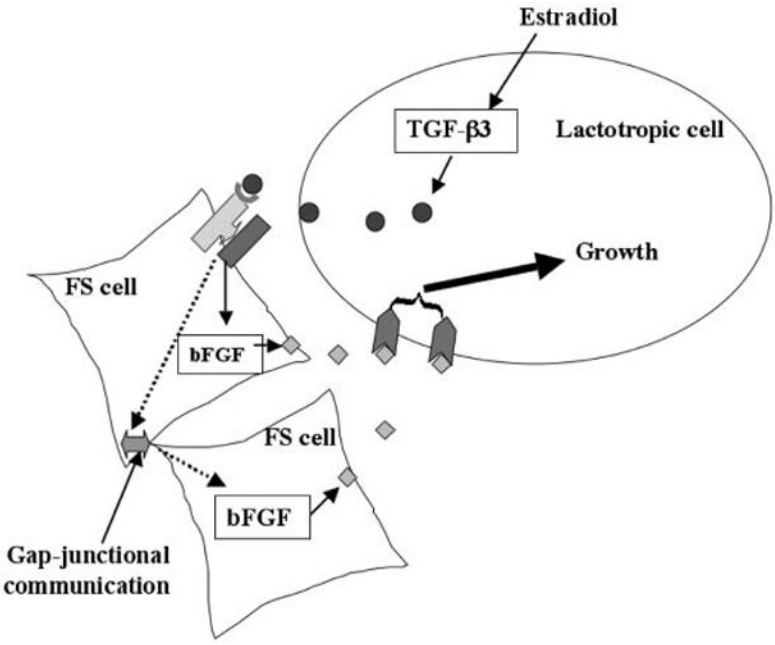

Fig. 7.

A diagram summarizing the postulated role of gap-junctional communication between FS cells in estradiol-induced prolactinoma. Estradiol exposure elevates TGFβ3 secretion from lactotropic cells that increases bFGF production from FS cells by activating TGFβ receptor signaling and by increasing gap-junctional communication between FS cells. The increased release of bFGF induces lactotropic cell growth and tumors.