TGF-β1 and fibroblast growth factor-1 modify fibroblast growth factor-2 production in type II cells

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Li, Cheng-Ming, Jody Khosla, Ines Pagan, Paul Hoyle, and Philip L. Sannes. TGF-β1 and fibroblast growth factor-1 modify fibroblast growth factor-2 production in type II cells. Am J Physiol Lung Cell Mol Physiol 279: L1038–L1046, 2000.—Fibroblast growth factor (FGF)-2, which stimulates DNA synthesis by type II cells in the lung, has been shown to be regulated by transforming growth factor (TGF)-β1, an important inflammatory cytokine, in vascular epithelium. The goal of this study was to determine if FGF-2 production by alveolar type II cells is modulated by TGF-β1 or FGF-1, which also stimulates DNA synthesis by type II cells. Isolated rat type II cells were exposed to 0–40 ng/ml of TGF-β1 or 0–500 ng/ml of FGF-1 in serum-free medium for 1–5 days. With a specific immunoassay, significant increases of FGF-2 protein in type II cell lysates to levels above those in control cells were achieved after 1 day of exposure to 100 ng/ml of FGF-1 and after 3 days of treatment with 8 ng/ml of TGF-β1. Similarly, transcripts for FGF-2 were dramatically increased above those in control cells with TGF-β1 or FGF-1, as were those for FGF receptor-1. These results demonstrate important regulatory links between FGF-2 and both TGF-β1 and FGF-1 in the alveolar epithelium that could contribute to the regulation of normal cell turnover, development, and the repair processes after injury in the lung.

Basic fibroblast growth factor; acidic fibroblast growth factor; alveolar epithelium; alveolar injury; pulmonary fibrosis; transforming growth factor

NORMAL LUNG FUNCTION depends on an intact alveolar epithelium, which is composed of alveolar type I and type II cells. The latter, in addition to regulating of surfactant metabolism and ion transport, functions to maintain stable cell populations under homeostatic conditions and to restore the integrity of the alveolar epithelium after alveolar epithelial damage by their capacity to proliferate and differentiate (1, 7). Numerous growth factors, including members of the fibroblast growth factor (FGF) family (17, 20), hepatocyte growth factor (20), transforming growth factor (TGF)-α (25), and epidermal growth factor (21, 25), have been found to stimulate DNA synthesis by type II cells or growth of fetal rat pulmonary epithelium. Among these, FGF-2 is particularly interesting because it has multiple biological activities in vivo and in vitro, including angiogenesis, mitogenesis, cellular differentiation, and repair of tissue injury (4). In the lung, FGF-2 has been immunolocalized in isolated alveolar type II cells (29) and in basement membranes including those in the alveolus (28). Little is known of its regulation in the lung, but its spectrum of effects and localization make it a likely focus for autocrine/paracrine linkage(s) between the growth factors produced by alveolar macrophages, alveolar epithelium, and fibroblasts and the modulation of type II cell behavior.

TGF-β and its isoforms have been shown to play multiple roles in regulating growth and differentiation in various cells and tissues, especially after injury (23). In the pulmonary alveolus, TGF-β has been shown to be produced by local activated macrophages, and increased levels have been detected in lung lavage fluid after lung injury (32). A recent study (18) showed that TGF-β stimulates the production of selected extracellular matrix (ECM) components by alveolar type II cells. In lung fibroblasts, TGF-β alone can increase expression of FGF-2 transcripts but not of protein (12). A specific relationship between TGF-β and FGF-2 has not been established in alveolar epithelial cells.

FGF-1 and -2 are closely related growth factors with 55% homology (2). They both have a broad spectrum of tissue distribution, target cells, and biological activities. The roles of FGF-1 and -2 are well understood (15), and a previous study (17) has demonstrated that both can stimulate DNA synthesis in isolated type II cells. A recent study (13) showed that exogenous FGF-2 can stimulate FGF-1 production in retinal pigmented epithelial cells. FGF-1 can induce gene expression of platelet-derived growth factor and nerve growth factor genes (8) but has yet to be linked to any regulation of FGF-2. The potential for growth factor interactions in the distal lung is further supported by the immunodetection of FGF-1 in type II cells (28) and macrophages (3) in normal lungs and by the expression of FGF-1 protein (3, 28) and mRNA transcripts (3) in alveolar epithelial cells and macrophages after injury. Because TGF-β1 and FGF-1 are found in injured lung (3, 32) and FGF-2 is a stimulant of DNA synthesis
in isolated type II cells, we postulated that TGF-β1 and/or FGF-1 could act indirectly in epithelial repair through the modulation of FGF-2 production in type II cells. To test this hypothesis, primary cultures of isolated adult rat alveolar type II cells were treated with different concentrations of TGF-β1 and/or FGF-1 at selected times postisolation, and the changes in FGF-2 protein expression and mRNA were examined. The results indicated that FGF-2 expression by isolated rat type II cells can be modulated by TGF-β1 and FGF-1.

MATERIALS AND METHODS

Cell Preparation

Briefly, rat type II pneumocytes were isolated from pathogen-free, 200- to 250-g Fischer CDF rats (Charles River Laboratories, Wilmington, MA) according to the procedure of Dobbs (7) with some minor modifications (31). Isolated cells were suspended in a concentration of 100,000 cells/ml with DMEM supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 50 μg/ml of gentamicin.

Cell Culture and Treatment

Standard culture dishes, 24-well plates, or chamber slides (Nunc, Naperville, IL) were coated with 0.06 g/ml of collagen (Sigma, St. Louis, MO) as described previously (31). The suspended cells were seeded at a density of 2 × 10^4 cells/cm^2 and allowed to attach and spread overnight at 37°C in 5% CO2. After 24 h, the attached cells were washed once with DMEM and exposed to serum-free, hormonally defined medium, treated cells were washed and processed as described previously (22). Radioactivity of the cell lysates was measured with an LKB 1219 scintillation counter (Wallac, Turku, Finland). Results were analyzed as previously described (31).

RT-PCR

RNA preparation. Type II cells grown in culture dishes were quickly washed once with cold 1× PBS, lysed, and extracted with TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the instructions of the manufacturer. After extraction, RNA samples were treated with RQ1 DNase (Promega, Madison, WI) at 37°C for 30 min to remove residual DNA and extracted again with TRI Reagent. The RNA was quantified by GeneQuant (Pharmacia, Piscataway, NJ), and its integrity was confirmed by electrophoresis on a denatured agarose gel containing formaldehyde (27).

Preparation of primers and competitive template standards. Based on gene sequences of rat β-actin, FGF-2, FGF receptor (FGFR)-1, and FGFR-2, the paired conventional forward and reverse primers and competitive template (CT) primers (Table 1) were selected with Oligo software and synthesized by Retrogen (San Diego, CA). CT primers were designed according to previous strategies (5a). Each was ~40 nucleotides in length, with ~20 nucleotides in the 5′-end identical to the corresponding conventional reverse primers and ~20 nucleotides in the 3′-end corresponding to an internal region of the opposite strand of the PCR target sequence.

Table 1. Primers used for PCR amplification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
<th>Position, nucleotides</th>
<th>Product, bp</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF-2</td>
<td>Forward</td>
<td>5′-AAGCGGCTCTACTGCAAG-3′</td>
<td>338–425</td>
<td>337</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5′-CAGGGCCCGTTGGTGATCCG-3′</td>
<td>674–655</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>R*-ACTGCGAGGCTTCAAAAGA-3′</td>
<td>565–547</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>FGFR-1</td>
<td>Forward</td>
<td>5′-GCGATACGACACCTTCTCCG-3′</td>
<td>326–347</td>
<td>777</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5′-GCTGCTGACGGTGAGGTG-3′</td>
<td>702–684</td>
<td>220</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>R*-ATT TCA CGGTCTCTCGGAGC-3′</td>
<td>526–508</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR-2</td>
<td>Forward</td>
<td>5′-GACACAGATAGCTTGCTGACG-3′</td>
<td>488–508</td>
<td>435</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5′-TGGGCTGGCGATCATA-3′</td>
<td>942–925</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>R*-TTGCGTTTGTGTGATGGGA-3′</td>
<td>765–746</td>
<td>284</td>
<td>35</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward</td>
<td>5′-GACCTTTGACACCCCCCAGCG-3′</td>
<td>2166–2186</td>
<td>392</td>
<td>19</td>
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<td></td>
<td>Reverse</td>
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<td>2557–2538</td>
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<tr>
<td></td>
<td>CT</td>
<td>R*-CCAGCTGCGGCTAGGAT-3′</td>
<td>2382–2366</td>
<td></td>
<td></td>
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</tbody>
</table>

FGF, fibroblast growth factor; FGFR, FGF receptor; CT, competitive template; R*, reverse primer sequence that was included at the 5′-end of the CT primer.
CT standards were synthesized and quantified as described by others (34). Briefly, the forward primer and CT primer of each target gene were used to amplify CT standards from cDNA known to express the relevant gene by standard PCR. Generated CT standards were purified with Wizard PCR Prep (Promega), quantified by GeneQuant and gel electrophoresis, and compared with pGEM size markers (Promega). The molarity of each CT standard was calculated as described by Willey et al. (34). Because the 3’ sequence of the designed CT primer corresponded to internal regions of the conventional PCR target sequence and the 5’-end to the conventional reverse primer sequence, the synthesized CT standards were shorter than the conventional PCR target fragments and could be further amplified by PCR with conventional forward-reverse primers. Therefore, they were competitively amplified with native genes when added to a conventional PCR, and the copy number of native genes was determined according to the amount of CT standard added and the ratio of native gene product and CT product (34).

RT reaction and quantitative PCR assay. Two micromgrams of total RNA from each sample were used for cDNA synthesis. The RT reaction was carried out as described previously (37) with random hexamer primers and AMV reverse transcriptase (Promega). RT products were stored at −20°C for later use.

The quantitative PCR assay was done with the QIAGEN Taq PCR core kit (QIAGEN, Chatsworth, CA). The assay volume of 50 μl consisted of 1 μl of forward and reverse primers (50 ng/μl), 5 μl of 10× PCR buffer, 10 μl of Q buffer, 0.2 μl of Taq polymerase (10 U/μl), 2 μl of 10 mM deoxynucleotide triphosphates, 5 μl of RT product, 5 μl of diluted CT standard, and 21.8 μl of 1,2-dihexadecyl-sn-glycero-3-phosphocholine-H2O. After being heated to 94°C for 4 min, reactions were cycled 40 times, with each cycle consisting of 1 min at 95°C, 30 s at 56°C, and 1 min at 72°C, followed by a final reaction at 72°C for 10 min. The PCR products were visualized by gel electrophoresis. To optimize the assay conditions, the appropriate amount of each CT standard was determined by a prerun quantitative PCR with serial 10-fold diluted CT standards against a fixed amount of normal type II cell cDNA. The appropriate amount of diluted CT standard, which gives a nearly equal CT product and native gene product, was used in the final assay. All of the PCR products from quantitative PCR assays were analyzed by 1.5% agarose gel electrophoresis and quantified by digital image analysis (Mocha Software, Jandel Scientific, San Rafael, CA).

Native gene transcripts in each sample were calculated according to the amount of CT standard added and the ratio of native gene product and CT product (34).

Immunohistochemical Analysis of FGF-2

Type II cells on culture slides were fixed with 5% formaldehyde-PBS for 10 min at room temperature. After they were washed, the slides were reacted with 2% horse serum-PBS for 30 min at room temperature and then incubated with rabbit anti-FGF-2 polyclonal antibody (1:500; Sigma) at 4°C overnight. As a control, the same concentration of normal rabbit serum was used. Twenty-four hours later, the attached cells were observed as isolated cells or as clusters of up to 10 cells (at ~30–40% confluence). Approximately 90–95% of the cells were confirmed to be type II alveolar cells as indicated by the abundance of typical lamellar bodies in their cytoplasm. After a change to serum-free medium, cells gradually grew to 50–60% confluence by 24 h (day 1) and 90–100% confluence by 72 h (day 3).

Immunofluorescent localization of FGF-2 protein in type II cells was proven in both serum-free and growth factor-treated type II cells, although FGF-1- and TGF-β1-treated cells tended to have a higher degree of reactivity (Fig. 1, a–c). Immunofluorescence was not detectable in specimens treated with normal serum (Fig. 1d). FGF-2 protein was also confirmed in cell lysates, but not in conditioned medium, by ELISA assay, which demonstrated that type II cells cultured in serum-free medium without growth factor stimulation produce ~40 pg FGF-2/μg protein.

Modulation of FGF-2 Production

After preliminary tests, dose-response experiments were conducted for 24 h (1 day) for FGF-1 and 72 h (3 days) for TGF-β1; then the time-course study was done with 8 ng/ml of TGF-β1 and/or 100 ng/ml of FGF-1 according to the results of the dose-response experiments. Both cell lysate samples and conditioned culture medium samples were analyzed for FGF-2 content by ELISA. Assay results of type II cell lysate samples showed that both TGF-β1 and FGF-1 treatments stimulated FGF-2 production in isolated rat type II cells in a dose-dependent fashion (Figs. 2 and 3). The maximum response to TGF-β1 was achieved with 8 ng/ml on day 3 (Fig. 2) and with 100–500 ng/ml for FGF-1 on day 1 (Fig. 3). Dose dependency was demonstrated by the lack of an effect on FGF-2 content in cultures that received <0.5 ng/ml of TGF-β1 or 1 ng/ml of FGF-1, whereas cultures that received 0.5–8 ng/ml of TGF-β1 and 10–100 ng/ml of FGF-1 showed sharp increases in FGF-2 protein in type II cells. A time course study demonstrated that treatment with 8 ng TGF-β1/ml increased FGF-2 to 76.7 ± 10.8 pg/mg protein on day 3 (181% of untreated control cells; Fig. 4) and that FGF-2 remained elevated on day 5. FGF-2 production by cells treated with 100 ng/ml of FGF-1 peaked on day 1 with

Data Analysis

All experiments were performed in triplicate or quadruplicate and were repeated at least three times. Statistical means ± SE were developed for the various treatment groups. Data were also expressed as percent of control (untreated or growth factor-free medium) so that %control = (mean value of treatment/mean value of untreated control) × 100. Each experimental group was compared with its control group by paired t-test. Differences were considered significant at P < 0.05.

RESULTS

Type II Cell Culture and Its FGF-2 Production

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197 ± 34 pg/mg protein (537% of untreated control cells) and then steadily declined thereafter as seen on days 3 and 5 (Fig. 4). However, growth factor-treated type II cells still produced substantially greater amounts of FGF-2 than nontreated cells on day 5 (FGF-1 treatment) and on day 7 (TGF-β1 treatment; data not shown). On day 1, the FGF-2 levels were 40 ± 6.3 pg/mg protein for TGF-β1-treated cells and 36.7 ± 6.4 pg/mg protein for untreated control cells, respectively (Fig. 4). When 8 ng of TGF-β1 and 100 ng of FGF-1 were combined to treat type II cells, the highest yield of FGF-2 was 188 ± 40 pg/mg protein (day 1), which was not statistically different from FGF-1 alone but was higher than TGF-β1 alone (Fig. 4).

In all conditioned culture medium samples, FGF-2 was not detectable by the ELISA assay used. This could have been a result of either the sensitivity of the ELISA kit (0.5 pg FGF-2/ml minimum) or the low level of free FGF-2 present in the medium.

The biological effects of TGF-β1 and FGF-1 were further examined by changes in total protein amount and thymidine incorporation into DNA. Results indicated substantial increases in thymidine incorporation (189% of control level on day 1 and 274% on day 2 for TGF-β1; 367% of control level on day 1 and 671% on day 2 for FGF-2; Fig. 5). Similarly, total cellular protein was increased (145% of control level...
for TGF-β1 and 173% of control level for FGF-1 on day 2; Fig. 6).

**TGF-β1 and FGF-1 Modulation of FGF-2 and FGFR-1 Gene Expression**

RT-PCR indicated that the abundance of mRNA for these genes is quite different. When 20 ng of total RNA from untreated type II cells were subjected to conventional RT-PCR analysis, the minimum number of cycles required for detection of selected mRNAs was 23 for β-actin, 30 for FGFR-2, 32 for FGFR-1, and 38 for FGF-2. Quantitative RT-PCR demonstrated that in 20 ng of total RNA of untreated type II cells, mRNA copy numbers were 560 ± 27.5 for FGF-2, 107,212 ± 8,203 for FGFR-1, 251,831 ± 14,278 for FGFR-2, and 25,775,000 ± 842,000 for β-actin.

Quantitative RT-PCR analysis demonstrated that both TGF-β1 and FGF-1 treatments can substantially increase the expression of FGF-2 mRNA and FGFR-1 mRNA in type II cells (Figs. 7 and 8). The response to both growth factors was very rapid at the mRNA level. The expression of FGFR-1 and FGF-2 mRNAs peaked within 24 h after treatment. By comparison, FGFR-1 mRNA expression peaked earlier than FGF-2 mRNA and declined to normal levels after 24 h. However, FGF-2 mRNA levels peaked at 24 h and returned to normal levels after 72 h.

As shown in Figs. 7 and 8, the largest increases in FGF-2 mRNA were 940 ± 10.2% of untreated control level for TGF-β1 and 1,310 ± 67% for FGF-1 after 1 day. The largest increase of FGFR-1 mRNA was 500% in both growth factor treatment groups after 12 h of treatment. However, no significant changes in FGFR-2 mRNA were observed in either group of growth factor-treated cells (data not shown).

**DISCUSSION**

Type II cell proliferation and differentiation are crucial processes in normal alveolar epithelial turnover, development, and repair after injury. The events that modulate these processes are poorly understood but likely involve a variety of key growth factors produced by type II and other lung cells. Previous studies by Sannes and colleagues (28, 29) suggested that FGF-2, which stimulates thymidine incorporation by type II cells, is present in both isolated type II cells and the basement membranes of the lung. The purpose of this study was to define specific conditions that modify FGF-2 production by type II cells.

TGF-β is a multifunctional factor that plays a central role in the regulation of cell growth and differentiation, having either stimulatory or inhibitory effects depending on the particular cellular context of its action. It has been shown to be mitogenic for osteoblasts and Schwann cells but to inhibit proliferation of lymphocytes and endothelial cells (23). Previous reports indicated that TGF-β inhibited DNA synthesis in neonatal and adult rabbit type II cells (25) but not in rat
type II cells (33). TGF-β has been shown to be linked to FGF-2 in two distinct ways: 1) FGF-2 has been shown to activate latent TGF-β (tissue bound) through its regulation of plasminogen activator and its inhibitor in vascular endothelium (9) and 2) TGF-β has been demonstrated to directly increase the expression of FGF-2 in lung fibroblasts (12). Such a relationship could be highly relevant to the alveolar epithelium in which the progenitor cells (i.e., type II cells) respond to FGF-2 as a mitogen. Previous studies have demonstrated that TGF-β stimulated biosynthesis of ECM in alveolar type II cells (18) and the gene expression of FGF-2 in lung cells (12). Given this profile, we reasoned that TGF-β could act to modulate other type II cell responses to produce certain growth factors. FGF-2 was a compelling target because of its known effects on DNA synthesis in type II cells, which is a key element in the reepithelialization of the alveolus after injury (1, 14). Accordingly, we studied the effects of TGF-β1 and FGF-1 on the production of FGF-2 and its receptors in vitro by examining the expression of FGF-2 and FGFRs.

Fig. 7. TGF-β1 and FGF-1 stimulate FGF-2 gene expression in rat alveolar type II cells. For quantitative RT-PCR detection of FGF-2 mRNA, equal amounts of FGF-2 internal control fragments [395 FGF-2 competitive template (CT) molecules] were included in each reaction. Results show higher amounts of native FGF-2 mRNA product in the growth factor-treated cell group (+) on day 1 than CT products compared with those in an untreated cell sample (−) (A and B). Quantitative digital image analysis confirmed that TGF-β1 and FGF-1 treatment increased FGF-2 mRNA copy number to as much as 940 and 1,310%, respectively, of control values after 1 day of exposure (C). After 1 day, FGF-2 mRNA transcripts were 3,301 ± 172 copies/10⁷ β-actin copies in FGF-1-treated cells and 2,688 ± 272 copies/10⁷ β-actin copies in TGF-β1-treated cells compared with 256 ± 43 copies/10⁷ β-actin copies in untreated control cells. Results are means ± SE; n = 3–7 experiments.

Fig. 8. FGF-1 and TGF-β1 upregulate FGF receptor (FGFR)-1 gene expression in rat alveolar type II cells. For quantitative RT-PCR detection of FGFR-1 mRNA, equal amounts of FGF-1 internal control fragments [395 CT molecules] were included in each reaction. Results show higher amounts of native FGFR-1 mRNA product in the growth factor-treated cell group (+) on day 1 than CT products compared with those in an untreated cell sample (−) (A and B). Quantitative digital image analysis confirmed that TGF-β1 and FGF-1 treatment increased FGFR-1 mRNA copy number to as much as 940 and 1,310%, respectively, of control values after 1 day of exposure (C). After 1 day, FGFR-1 mRNA transcripts were 3,301 ± 172 copies/10⁷ β-actin copies in FGF-1-treated cells and 2,688 ± 272 copies/10⁷ β-actin copies in TGF-β1-treated cells compared with 256 ± 43 copies/10⁷ β-actin copies in untreated control cells. Results are means ± SE; n = 3–7 experiments.

*P < 0.05 vs. control group.
through the detection of their specific mRNA and FGF-2 protein. The results indicated that alveolar type II cells possess the components of an FGF-2 autocrine loop (i.e., expression of FGF-2 and its receptor). Such a loop could be initiated by exogenous growth factors such as exposure to FGF-1 and/or TGF-β1, which could, in turn, lead to an increase in the mRNA level of FGF-2 and its FGFR-1. Under the conditions used in the present study, both TGF-β1 and FGF-1 were found to stimulate DNA synthesis as reflected in thymidine uptake and protein production and clearly acted to upregulate FGF-2 and FGFR-1 mRNA expression. Although it is not clear how much of the effect on DNA synthesis is direct or indirect (e.g., effects of FGF-2), the data support the notion that TGF-β1 is capable of influencing or modulating epithelial proliferative and reparative events after injury. This may be a key relationship that, in effect, defines the resolution of repair in alveolar regions. Furthermore, FGF-2 has been immunolocalized in the alveolar basement membrane (ABM) (28), and its detection is diminished 7 days after oxygen-induced fibrosis (29). Presumably this is due to its release from the ABM by proteolytic enzymes attendant to inflammation (26). Such release could be expected to be important in initial reepithelialization events in the alveolus as type II cells divide and differentiate into type I cells. As reparative processes continue, the presence of TGF-β in the alveolar environment could act on type II cells in two clearly distinctive ways, both involving biosynthesis of FGF-2 and FGFR-1: 1) facilitate end-stage repair by stimulating further reepithelialization and 2) stimulate biosynthesis of new ABM, including FGF-2 and other connective tissue components (30). In both cases, TGF-β would be acting to return alveolar structure-function relationships to normal.

Such mechanisms could be further promoted by additional growth factors known to be present and active in alveolar regions, such as FGF-1, which has a broad range of biological activity that includes modulation of growth factor production (8). However, its effect on members of its own growth factor family has not been widely appreciated. The present study showed that FGF-1 can modulate the production of FGF-2 protein and its FGFR-1 gene. These results, combined with previous data (13), indicate that there is a potentially important regulatory link between FGF-1 and -2, which demonstrates that growth factors in the same family can regulate each other to promote biosynthetic activity and proliferation. In this way, FGF-1 could act to amplify its own activity through upregulation of a powerful related mitogen and its receptors.

FGFs interact with their target cells via a dual-receptor system consisting of a cell surface heparan sulfate proteoglycan (low-affinity receptor) and a transmembrane receptor linked to tyrosine kinase (high-affinity receptor) (11). There are at least four types of receptors (FGFR-1 to -4) with intrinsic tyrosine kinase activity, all derived from separate genes (11). The mechanism for differential expression of these receptor genes has not been clearly defined. FGF-2 has been reported to bind to both FGFR-1 and -2 with high affinity (6). Endogenous FGF-1 has been shown to stimulate FGF-1 gene expression in retinal pigmented epithelium (13). In human liver myofibroblasts, TGF-β1 treatment stimulates both FGF-1 and -2 gene transcription (24). However, in the present study, the FGF-1 message level was increased with TGF-β1 or FGF-1 treatment. This difference suggested the possibility of a FGFR preference in different cell types and that in type II cells, FGFR-1 may play a more important role in FGF-2 binding and signal transduction. In support of this notion, whole animal studies on rats exposed to 85% oxygen indicated that expression of both FGF-2 and FGF-1 was elevated in lungs after 6 days of exposure (5). Because both TGF-β1 and FGF-1 are known to be elevated in models of alveolar damage and in injured lung (3, 32), it is possible that their presence, at least in part, accounts for the increased expression of FGF-2 mRNA and protein and FGFR-1 mRNA.

Many growth factors, including those of the FGF family, bind to components of the ECM (15). When tissue environments are damaged or altered during inflammatory events, these latent growth factors can be proteolytically liberated from the ECM and thus influence local proliferative and reparative events (9). It is not clear from the results of the present study whether the biosynthesized FGF-2 is actually released from type II cells. The FGF-2 produced by rat type II cells was only found in cell lysates (which would include ECM) but not in conditioned medium. This could be due to the low level of FGF-2 in the medium or the limitations of the assay (0.5 pg FGF-2/ml minimum). It could also be a result of the fact that at least some of the detectable FGF-2 was associated with biosynthesized ECM. This issue was not addressed by the current work but could be resolved by immunolocalization of FGF-2 at the level of the confocal or electron microscopy or by appropriate biosynthetic labeling studies.

The increase in DNA synthesis observed in type II cells treated with TGF-β1 was somewhat surprising, given the results of previously published studies (25, 33). It has been demonstrated that manipulation of experimental conditions can elicit dramatically different responses from isolated type II cells (25). For example, insulin-like growth factor I increased type II cell numbers when the cells were plated at high density but had no effect at lower density. FGF-1, on the other hand, has both stimulatory and inhibitory effects on human type II cells depending on the serum concentration in the culture medium. In addition to the obvious species and age differences when studies are compared, the more subtle differences such as cell density, serum concentration, or the matrix substrata on which the cells are cultured can greatly affect which growth factors elicit a type II cell response and its magnitude (25). It is possible that the lower cell density cultures (2 × 10^4 cells/cm^2) used in the present study were necessary to achieve the result of DNA synthesis in adult rat type II cells, whereas the higher density cell cultures of neonatal rabbit type II cells (25) did not.
contrast, low cell density alone may not be sufficient to facilitate a TGF-β1-stimulated DNA synthesis as evidenced in earlier work (25) with lower cell densities than those employed here. Interestingly, Ryan et al. (25) used uncoated plates in their culture conditions, so it could be argued that substrata could play an equally important role. Although this discussion does not address the distinction between DNA synthesis and cell proliferation in type II cells, it does emphasize the importance of those conditions, probably multiple in nature, which favor the capacity of TGF-β1 to stimulate FGF-2 production, which, in turn, could account for the observed increase in DNA synthesis. In vivo, this could translate into modest but potentially important increases in type II cell numbers during repair after injury.

In conclusion, the data presented here demonstrate that TGF-β1 and FGF-1 stimulate gene and protein expression of FGF-2 and its receptor in type II cells in vitro. However, in vivo, the presence of TGF-β1 and FGF-1 after injury or during an inflammatory event could modulate type II cells to increase the expression of FGF-2 and its receptor, which in turn, could act as an autocrine or paracrine growth factor to increase type II cell numbers. Such a relationship could constitute a significant mechanism for influencing proliferative and biosynthetic events that are critical for the proper resolution of tissue injury in the alveolus.

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