Bronchial epithelial cell growth regulation in fibroblast cocultures: the role of hepatocyte growth factor

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Skibinski G, Elborn JS, Ennis M. Bronchial epithelial cell growth regulation in fibroblast cocultures: the role of hepatocyte growth factor. Am J Physiol Lung Cell Mol Physiol 293: L69–L76, 2007. First published March 23, 2007; doi:10.1152/ajplung.00299.2006.—Proliferation of bronchial epithelial cells is an important biological process in physiological conditions and various lung diseases. The objective of this study was to determine how bronchial fibroblasts influence bronchial epithelial cell proliferation. The proliferative activity in cocultures was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and direct cells counts. Concentration of cytokines was measured in cell culture supernatants by means of ELISA. In primary cell cocultures, fibroblasts or fibroblast-conditioned medium enhanced 1.85-fold the proliferation of primary bronchial epithelial cells (P < 0.02) compared with bronchial epithelial cells cultured alone. The proliferative activity in cocultures and in fibroblast-conditioned medium was reduced by neutralizing antibody to hepatocyte growth factor (HGF) and HGF receptor c-met. Neutralizing antibodies to FGF-7 and IGF-1 had no effect. Treatment of fibroblast-epithelial cocultures with anti-IL-6 and anti-TNF-α neutralizing antibodies and with indomethacin decreased production of HGF. These results indicate that cytokines and PGE2 may indirectly mediate epithelial cell proliferation via the regulation of HGF in bronchial stromal cells and that HGF plays a crucial role in proinflammatory cytokine-induced proliferation in the experimental system studied.

EPITHELIAL MESENCHYMAL INTERACTIONS control epithelial growth and differentiation and regulate tissue homeostasis in the airways as well as in other organs. These interactions are based on three fundamental processes: production of soluble factors with paracrine and/or autocrine activities, cell-matrix interactions, and signaling by direct cell contact. Proliferation of airway epithelial cells is a crucial step for recovery from mucosal injury, and it is likely to be modulated (both negatively and positively) by various growth factors and cytokines (5, 18, 19, 32). Despite increasing information about factors affecting airways epithelial cell proliferation, the understanding of mechanisms regulating airway epithelium growth is incomplete. In particular, the role of other cell populations commonly present in close proximity to the regenerating epithelial cells is not well understood. It is probable that like the growth and differentiation of hematopoietic cells depends on stromal cells, growth of airway epithelial cells is also modulated by other cell populations underlying the epithelium.

During inflammatory respiratory disorders, extensive injury of airway epithelium may occur with shedding of sheets of damaged cells in the bronchial or alveolar lumen but also with activation of the surviving epithelial cells and of the underlying fibroblasts. The ability of epithelial cells to repair a wound is dependent not only on their activities and their interaction with the matrix, but also on the cytokine milieu and therefore on the interactions with other parenchymal cells present in the airways, e.g., bronchial wall fibroblasts, which secrete cytokines able to influence epithelial cell function (41). The connective tissue is known to have a general supporting effect for the overlying epithelium, and fibroblast cell lines (embryonic mouse and human) (16) as well as primary fibroblasts (43) have been used as feeder layers in different in vitro models.

Several growth factors and interleukins have been detected in airways as well as in bronchial fibroblasts (BF) and bronchial epithelial cell (BEC) cultures such as interleukin-8 (IL-8), IL-6, granulocyte monocyte colony-stimulating factor, tumor necrosis factor-α (TNF-α) and -β (TNF-β), platelet-derived growth factor (PDGF), NGF, insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and several members of the fibroblast growth factor (FGF) family (12, 19, 32). The epithelial-specific growth factors such as HGF, FGF-7, IGF-1 are especially important mediators of mesenchymal-epithelial interactions during lung development, lung inflammation, and lung repair (10, 31, 42). HGF was originally identified and cloned as a potent mitogen for mature hepatocytes and is now recognized as the most potent of epithelial mitogens (23, 25, 27).

Recently, it was shown that postmitotic fibroblasts, although irreversibly blocked from proliferation by X-irradiation or mitomycin C treatment, are still functionally active to constitutively express cytokines and even more so react to external stimuli by modulating their mRNA and protein expression (21, 43).

In this study, we determined the mechanisms underlying the supportive role of BF on BEC proliferation. By using postmitotic fibroblasts, we intended to eliminate proliferation effects induced by epithelial cells on fibroblasts. We show that fibroblasts regulate epithelial cell proliferation predominantly through paracrine action. We also demonstrate that, in our model, HGF is an important paracrine factor produced by fibroblasts that promotes BEC proliferation. These controlled interactions may also be functional in vivo and allow a rapid induction of cell proliferation in repair process of bronchial epithelium.
FIBROBLASTS STIMULATE BRONCHIAL EPITHELIAL CELL GROWTH

MATERIALS AND METHODS

Reagents. The following neutralizing polyclonal antibodies purchased from R&D Systems (Abington, United Kingdom) were used: goat anti-human keratinocytic growth factor/FGF-7 (cat. no. AF-251-NA), goat anti-human HGF (cat. no. AF-294-NA), goat anti-human IGF-1 (cat. no. AF-291-NA), IL-6 (cat. no. AF-206-NA), anti-TNF-α (cat. no. AF-210-NA), and anti-c-met (cat. no. AF276). Normal goat IgG (cat. no AB-108-C, R&D Systems) served as a negative control with neutralizing antibodies. Monoclonal antibodies against α-smooth muscle actin (ab7817), vimentin (ab7752), pan-cytokeratin (ab7753), cytokeratin 5 + 8 (ab9005), cytokeratin 18 (ab7798), and CD31 (ab24590) were from Abcam (Cambridge, England). Recombinant human HGF was purchased from R&D Systems and was activated with human serum according to the manufacturer’s protocol. Transwell membranes (pore size 0.4 μm) were from Costar-Corning (Cambridge, England). Vitrogen collagen was used for coating plastic surfaces (Cohesion Technologies, Palo Alto, CA). All other reagents except those used for cell culture listed below were from Sigma (Poole, England).

Cell culture. Primary BEC were derived from lung samples obtained from 10 patients undergoing lung surgery for removal of a primary localized lung tumor. Normal tissue from noninvolved segments, remote from the solitary lesion, was obtained. Cells were prepared as described by Fulcher et al. (8). Briefly, excised airways, from which excess connective tissue had been removed, were rinsed in cold DMEM (Invitrogen, Paisley, United Kingdom) plus antibiotics and then incubated in 0.1% protease (Sigma type XIV) for 16 h at 4°C. Ten percent serum was added to neutralize the protease, and cells were freed by gentle scraping and agitation. The cells were collected by centrifugation at 150 g, seeded into collagen-coated flask (Orange Scientific, Braine-L’Alleud, Belgium), and cultured at 37°C in fully humidified atmosphere containing 5% CO₂ with a serum-free bronchial epithelial growth medium (Promocell, Heidelberg, Germany) and containing bovine pituitary extract, hydrocortisone, human epidermal growth factor, epinephrine, transferrin, insulin, retinoic acid, and triiodothyronine. Cells grown in submersion cultures were used at passage 3. They stained positively with pan-cytokeratin, cytokeratin 5 + 8, and cytokeratin 18 and negative with anti-vimentin, anticytokeratin 13, and anti-CD31 antibodies.

To obtain fibroblasts, a small section of bronchus was resected from the nondiseased portion of a lobe from a human lung obtained at thoracotomy from patients undergoing surgery for the removal of lung tumors. Small 2-mm² explants were plated out in 10-cm diameter petri dishes, and outgrowing fibroblasts were trypsinized and passaged in DMEM containing 10% FBS supplemented with antibiotics (50 μg/ml streptomycin and 50 μ/ml penicillin) as described by Matsushima et al. (22). These cells were used between passages 4 and 8 and showed typical fibroblast morphology. On immunohistochemical examination, they stained positively with anti-vimentin antibodies; 5% of cells or less were positive for smooth muscle actin and negative with anti-cytokeratin and anti-CD31 antibodies, indicating that the cultures did not contain epithelial or endothelial cells. Fibroblasts from human lymph nodes and skin were obtained as described previously (38). This study was approved by the ethics committee of the Queen’s University Belfast, and all patients provided written informed consent.

Cultures of BEC with BF. Fibroblasts (0.3 × 10⁵ cells per well) were plated in 24-well dishes and grown in DMEM containing 5% FBS for 2 days. After 2 days, cells (~80% confluent) were treated with mitomycin C (6 μg/ml for 8 h) to inhibit fibroblast growth. After mitomycin treatment, the fibroblasts were washed with PBS. Epithelial cells were plated onto the fibroblast monolayers at 8 × 10⁴ cells per well and cultured for 6 days in medium consisting of RPMI 1640 supplemented with 2% FBS, 2 mM L-glutamine, 0.5 ng/ml epidermal growth factor, 5 μg/ml transferrin, 5 μg/ml insulin, 30 nM triiodothyronine, 5.5 μM epinephrine, 100 U/ml penicillin, 100 μg/ml streptomycin (hereafter referred to as growth medium). At the end of culture period, the medium was removed, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added (200 μl), and the medium was incubated for 8 h. The formazan product was extracted with SDS-dimethyl formamide solution, 100-μl aliquots were transferred to 96-well plates, and absorbance was measured in a plate reader as described below. For separated coculture experiments, fibroblasts and epithelial cells were cultured without any cell-to-cell contact by using a Transwell insert (pore size 0.4 μm; Transwell Clear, Costar-Corning) coated with collagen (Vitrogen). As initial experiments showed no significant differences between syngeneic and allogeneic combination of fibroblasts and epithelial cells, cells originating from different subjects were used throughout most of the study.

Preparation of conditioned culture medium from BF. Fibroblasts were plated in T-25 flask in 5% FBS DMEM and allowed to reach 80% confluence. The cultures were then rinsed twice and cultured in serum-free growth medium, and conditioned medium was collected 48 h later. Antibody neutralization of conditioned medium was performed at 37°C for 1 h before its addition to epithelial cell cultures.

Colometric MTT assay. Cell proliferation was evaluated by a colorimetric MTT assay. In brief, the Transwell inserts were transferred to new 12-well plates, and 150 μl of MTT solution was added to the upper part of the Transwell and incubated for 8 h. The blue formazan product was extracted with an SDS-dimethyl formamide mixture, 100 μl of the solution was transferred to 96-well plates, and absorbance was measured at a wavelength of 570 nm with a background reading at 660 nm on a spectrophotometric plate reader (VersaMax tunable microplate reader, Molecular Devices).

Matrix factors. Matrices of extracellular factors were prepared by culturing the fibroblasts until they were 70% confluent. The cultures were washed three times with PBS and then cultured for 2–3 days in serum-free medium removing the cells with nonenzymatic detachment solution (Sigma) or 0.02% EDTA at 37°C as described by Krtolica et al. (20). Epithelial cells were seeded directly on such matrices.

Determination of cytokine concentrations. Measurement of IL-6 in cell culture supernatant was performed using PeliKine Compact ELISA kits (Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands). TNF-α and HGF measurements in culture supernatants were carried out using DuoSet ELISA kits from R&D Systems.

Statistical analysis. All data are presented as the means ± SE unless otherwise noted. Differences between groups were analyzed using nonparametric Mann-Whitney U test and considered to be significant if P < 0.05. GraphPad Prism was used to plot graphs and to analyze the data.

RESULTS

Effect of BF on BEC proliferation in direct coculture. In the first experiments, we used the simplest version of coculture model of fibroblasts and BEC, the feeder-layer system, where epithelial cells together with fibroblasts on plastic dishes are grown submersed in culture medium. In order not to overgrow the epithelial cells, the fibroblast feeder cells had to be converted into an irreversible postmitotic state achieved by mitomycin treatment. Human BF were grown in 24-well plates until 80% confluent. Then they were treated with mitomycin C (6 μg/ml) to inhibit proliferation. Whereas untreated fibroblasts expanded threefold in 10–12 days, mitomycin-treated cells did not multiply but survived as attached cells for up to 6 wk. This number slightly declined during the first 2 days and at day 4 stabilized at ~80% of the initially attached cell numbers. This was confirmed by MTT assay, direct counting, and negative staining with Ki-67 antibody detecting a nuclear antigen.
known to be expressed in active phases of the cell cycle (data not shown). Normal human BEC obtained from the same donor were grown directly on top of the fibroblast monolayers. As shown in Fig. 1, direct coculture of BEC on top of mitomycin-treated fibroblast monolayers resulted in 1.84-fold increased epithelial cell proliferation as indicated by the MTT assay \((P < 0.01)\). Fibroblasts isolated from lymph nodes and skin, treated with mitomycin in the same way as BF, exhibited a less supportive effect for epithelial cell proliferation (1.26-fold increase and 1.57-fold increase, respectively; \(P < 0.05\)). These experiments show that BF stimulate epithelial cell growth (Fig. 1). The coculture of homologous epithelial and mesenchymal cells should be more relevant for studying the interaction between the two cell types. However, there was no difference in stimulation of epithelial cells growth when BF and BEC from the same or different subjects were used (data not shown).

**Contribution of fibroblast-secreted factors.** The observed interaction between fibroblasts and epithelial cells may be mediated either by secreted soluble factors or factors of the extracellular matrix. To determine the participation of components of extracellular matrix, fibroblasts were allowed to grow in serum-free medium for 3 days and deposit extracellular matrix onto the culture dishes. The cells were removed by calcium chelation using nonenzymatic detachment solution or 0.02% EDTA as described by Krtolica et al. (20). Immunocytochemistry showed that dishes contained abundant fibronectin, suggesting that at least this component survived cell removal (data not shown). Epithelial cells were then plated onto the matrices, and cell proliferation was quantified by the MTT assay. The cells were grown for 7 days. Control cells were seeded onto standard collagen-coated wells. Approximately 20% better growth was observed in epithelial cells cultured onto the matrix produced by BF \((P < 0.05)\). No significant difference was found in proliferation of epithelial cells cultured on extracellular matrix from skin and lymph node fibroblasts compared with commercial collagen (Fig. 2).

**Effects of BF on BEC proliferation in Transwell system.** To further investigate whether the effects of fibroblasts on epithelial cell proliferation depended on direct cell-to-cell contact, the experiments were carried out in Transwell system cultures where cells grow in close proximity separated by a permeable membrane. BEC were first seeded on collagen-coated Transwell membranes and allowed to attach for 24 h. After that period, the Transwells were placed into the wells of a 24-well plate containing 80% confluent and mitomycin-treated fibroblasts. The cocultures were allowed to grow for 6 days. As indicated in Fig. 3A, the proliferation of epithelial cells cocul-

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**Fig. 1.** The effect of bronchial, lymph node, and skin fibroblasts on bronchial cell proliferation in direct coculture is shown. Bronchial epithelial cells (BEC) were cultured alone or directly on top of mitomycin-treated bronchial fibroblasts (BF) in 12-well plates for 6 days. Cell proliferation was assessed by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described in MATERIALS AND METHODS. The results from BF epithelial cocultures are from 6 experiments. Results from experiments using lymph node and skin fibroblasts are from 4 experiments. Results are expressed as means ± SE \((n = 4–6); P < 0.02\). OD, optical density.

**Fig. 2.** Effects of fibroblast extracellular matrix on the growth of BEC are shown. Fibroblasts were grown in culture plates to ~70% confluence. Then, the cells were detached by incubation with nonenzymatic detachment solution. Plates were washed with serum-free DMEM and used in experiments. After 6 days, cell proliferation was assessed by MTT assay. Results of 4 experiments are expressed as means ± SE. LNF, lymph node fibroblast; SF, skin fibroblast. \(*P < 0.02.\) Control, cells grown on collagen-coated plates.

**Fig. 3.** The comparison of cell growth in Transwell cocultures vs. fibroblast-conditioned media (CM) is shown (means ± SE; \(n = 3\)). Epithelial cells were grown in the upper compartment of Transwell cultures, while in the lower compartment, BF were cultivated. In parallel, epithelial cells were grown in conventional collagen-coated plates in conditioned medium from BF cultures. After 6 days, proliferation was estimated by MTT assay (A) or direct cell count (B). \(*P < 0.02.\)
tured with fibroblasts was ~2.5 times higher than in epithelial cells cultured alone \((P < 0.05)\). In some experiments, instead of using the MTT assay to assess proliferation, epithelial cells were counted in the apical Transwell compartment after coculture with fibroblasts. The obtained results closely resembled those obtained with the MTT test (Fig. 3B).

Having established increased epithelial cell proliferation in the presence of fibroblasts in Transwell cocultures, the effects of conditioned media from BF were studied to confirm that the increase of epithelial cell proliferation in cocultures with epithelial cells was cell-to-cell contact independent. Epithelial cells were grown in the presence of conditioned medium obtained from serum-starved BF. As shown in Fig. 3, conditioned fibroblast medium had an effect similar to the coculture system (Fig. 3, A and B).

Effects of anti-growth factor antibodies on fibroblast-stimulated BEC proliferation. Having established that fibroblasts regulate BEC proliferation mainly through the production of soluble mediators, a series of experiments was performed using neutralizing antibodies against growth factors thought to influence epithelial cell proliferation. As indicated in Fig. 4A, the enhanced proliferation of epithelial cells in the presence of fibroblast-conditioned medium was significantly inhibited by the addition of neutralizing anti-HGF antibodies \((P < 0.02)\). Control IgG exerted no inhibitory effect on epithelial cell proliferation (data not shown). There was a small, statistically nonsignificant effect of anti-IGF-1 (used at 10 \(\mu\)g/ml) and anti-FGF-7 on cell proliferation compared with anti-HGF neutralizing antibodies (Fig. 4A). The effect of neutralizing antibody was dependent on antibody concentration (Fig. 4B). For anti-IGF-1 and anti-FGF-7 antibodies, increasing the concentration of antibodies to 50 \(\mu\)g/ml did not increase the degree of inhibition (data not shown). The antibody against HGF receptor c-met also significantly reduced the increased proliferation of BEC (Fig. 4C).

In separate experiments, BEC were cultured in the presence of increasing concentrations of human recombinant HGF. As shown in Fig. 4D, HGF induced dose-dependent increase of cell proliferation reaching maximal value at 50 ng/ml in agreement with results published previously (36, 40).

Production of HGF by BF upon coculture with BEC. To investigate the possible mechanism, the next experiments were performed using Transwells. Fibroblasts were grown to 80% confluence and were then treated with mitomycin and cultured in contact with epithelial cells placed in Transwells. Cultures were allowed to grow, and culture medium was collected after 6, 24, and 48 h. Media from epithelial cells cultured without fibroblasts and fibroblasts cultured without epithelial cells were used as controls. As shown in Fig. 5, fibroblasts on their own produced HGF in detectable amounts. However, the coculture of two cell types substantially increased concentration of HGF secretion. Epithelial cells on their own did not produce HGF (Fig. 5).

Fig. 4. \(A\): fibroblast-conditioned medium was treated with different neutralizing antibodies and then used to study the effect on epithelial cell proliferation. On day 6 of culture, MTT assay was performed. \(B\): inhibition of proliferation by anti-hepatocyte growth factor (HGF) neutralizing antibody depended on the dose of antibody used. Similar inhibitory effect was achieved by the use of neutralizing anti-c-met antibody (\(C\)). \(D\): BEC proliferation in response to human recombinant HGF after 6 days of culture. Results are presented as means ± SE \((n = 4)\). *\(P < 0.02\). BFCM, BF-conditioned medium.
Involvement of proinflammatory cytokines in HGF production. Substantial amounts of IL-6 and TNF-α accumulated in the supernatants of fibroblast and epithelial cell cocultures (Table 1). It is known that the HGF promoter region retains the responsive element for IL-6 and TNF-α (23). This suggests that IL-6 and TNF-α, as in other systems (15, 30), may contribute to the production of HGF by cocultures.

To explore this hypothesis further, we performed experiments using antibodies against these cytokines. Fibroblasts and epithelial cells were cocultured in the presence or absence of neutralizing anti-TNF-α and anti-IL-6. This resulted in a significant decrease in the concentration of HGF in culture supernatants. Addition of anti-TGF-β antibody had no effect on level of secreted HGF (Fig. 6A). Proliferation experiments performed in parallel showed inhibition of epithelial cell growth in the presence of anti-cytokine antibodies similar to anti-HGF antibody (Fig. 6B). Since both IL-6 and TNF-α have no direct effect on BE proliferation, the reduced IL-6 and TNF-α levels reduced proliferation by lowering HGF production in fibroblasts.

Involvement of PGE2 in HGF production. A growing body of evidence supports the role of PGE2 as one of the most potent HGF inducers. As PGE2 is produced in substantial amounts by BEC at both basal state and after stimulation (1, 2, 26), we hypothesized that in our system, PGE2 production may contribute to increased HGF secretion by fibroblasts and subsequent epithelial cells proliferation. The performed experiments showed that PGE2 synthesis inhibitor indomethacin introduced in coculture system significantly decreased HGF concentration in cultures supernatant in a dose-dependent manner (Fig. 7A). Proliferation experiments performed in parallel showed significant inhibition of epithelial cell proliferation indicating that PGE2 may be involved in regulation of epithelial cell proliferation via stimulation of HGF secretion (Fig. 7B).

**DISCUSSION**

The epithelium-fibroblast unit is in a key position to respond to and coordinate responses to environmental stimuli (12, 19). It is well established that homeostasis of epithelial organs such as skin, lung, and gut critically depends on interaction with the adjacent mesenchyme. The study of the molecular mechanisms of these interactions in vivo is complicated by too many variables and the lack of properly controlled experimental conditions. Therefore, in vitro models have been developed representing different coculture systems of epithelial and mesenchymal cells, the most simple one, the feeder-layer culture, described for BEC by Kitamura and coworkers in 1990 (16). The substantial proliferation of epithelial cells in coculture has been utilized since then, but the epithelial growth regulation had been rather obscure. Understanding the mechanism whereby epithelial cells and fibroblasts communicate may lead to novel targeted therapies for the treatment of asthma and other respiratory diseases that involve airway remodeling.

HGF, also known as scatter factor, was originally identified and cloned as a potent mitogen for mature hepatocytes and is
.now recognized as one of the most potent modulators of epithelial, endothelial, and myogenic cells (33, 36). More recently, HGF has also been implicated in the proliferation and differentiation of early hematopoietic progenitor cells and in monocyte-macrophage differentiation (36). Studies have shown that HGF is predominantly expressed in mesenchymal cells (18) and acts on epithelial and other cells through c-met/HGF receptor tyrosine kinase (39). In lung development, a paracrine loop between HGF-produced fibroblasts and c-met-expressing epithelium is involved in pulmonary branching and tubular formation (29). HGF production is upregulated in adult lungs in response to parenchymal injuries (46). Administration of anti-HGF IgG inhibited the lung repair in rodent models associated with the suppression of lung epithelial cells (33, 44). Inversely, supplementation of HGF leads to enhanced regeneration of bronchial and alveolar epithelial cells (28, 33, 35).

In this study, using a primary BF-BEC coculture model, we show that BF stimulate the proliferation of BEC, which is based on reciprocal modulation of cytokine and growth factor production in epithelial cells and fibroblasts. This was evident both in experiments where epithelial cells and fibroblasts were in direct contact and in Transwell experiments where cells grew separated by a permeable membrane. The stimulatory activity was mainly provided by conditioned medium. Cell-to-cell contact also provided some growth support for epithelial cells. It is postulated that in the airways as in other tissues (41), growth factor activity is likely to be regulated by sequestration and binding to molecules in the extracellular matrix. However, overall in this model, the extracellular matrix does not play a very significant role in the regulation of BEC proliferation. It cannot be excluded, however, that nonenzymatic treatment of fibroblasts to detach them from plastic destroys or modifies critical substances of the extracellular matrix responsible for growth regulation. It should be also noted that the paracrine effect on BEC proliferation was also exerted by fibroblasts derived from skin and human lymph node, although to a lesser extent. These results suggest that fibroblast-mediated paracrine effects on BEC are tissue nonspecific. Despite the apparent similarity of fibroblasts in different tissues, there is evidence that different phenotypes exist and that regional differences in wound healing may to some degree be caused by such phenotypic differences. For example, fibroblasts from buccal mucosa and periodontal ligament produce significantly more HGF than skin fibroblasts after coculture with keratinocytes (11). In our experimental system, BF performed better than skin and lymph node fibroblasts. Investigations into mechanisms of these effects are currently being carried out in our laboratory.

Although HGF effects have been widely studied in other organs, the mechanism by which HGF exerts its protective effects in the airways has not been thoroughly studied. The fact that neutralizing antibody against HGF can significantly reduce epithelial cell proliferation in epithelial-mesenchymal coculture shows that HGF plays an important role in mediating growth-promoting effects from lung. Neutralizing anti-HGF antibodies also dose dependently decreased the stimulatory effect of conditioned medium obtained from serum-starved fibroblasts on BEC proliferation. Obtaining similar effects with the blocking anti-c-met antibody provides even stronger evidence for HGF. The finding that HGF is a growth-promoting factor of BEC is in agreement with previous studies documenting growth-promoting effects for recombinant HGF on BEC (40, 42) and with results showing that HGF, by its multiple biological activities, is leading to normal tissue architecture (44). FGF-7 is a member of the FGF family, which in vertebrates includes 22 members and is produced by mesenchymal fibroblastic cells. It induces a variety of epithelial responses including proliferation, migration, and morphogenesis and is also implicated in epithelial repair process (42). Surprisingly, anti-FGF-7 neutralizing antibody, even if used in great excess, was not effective in inhibition of epithelial cell growth. However, in a recent study, FGF-7 has not been shown to stimulate repair of wounded human epithelial cell monolayer (45). The lack of effect of neutralizing anti-FGF-7 antibodies may be explained by redundancy FGF-7 receptor or existence of other ligands for the FGF-7 receptor, e.g., FGF-10 (14). IGF-1, although implicated in BEC proliferation (31), had no effect in our system. HGF is no doubt important but not the only mediator of enhanced epithelial cell proliferation caused by fibroblasts. Incomplete reduction of epithelial cell proliferation observed in our experiments supports such interpretation.

Induction of HGF and its receptor, c-met, have been reported in some systems. It was also reported that the epidermal growth factor, PDGF, and basic FGF markedly stimulated production of HGF in various systems (14). Therefore, it is possible that several cytokines regulate the production of HGF. In our study, neutralizing anti-TNF-α and anti-IL-6 antibodies significantly inhibited the coculture-induced production of HGF. Whereas the cytokine IL-6 and TNF-α themselves have no effect on

![Figure 7](http://ajplung.physiology.org/)

**Fig. 7.** The effect of different concentrations of indomethacin on HGF production (A) and on epithelial cell proliferation in BEC-BF cocultures (B). Results are presented as means ± SE (n = 4). *P < 0.02.
proliferation of BEC in monolayers, abrogation of their function in cocultures inhibited epithelial cell proliferation to the extent comparable to that obtained by neutralization of HGF. Similar effects of IL-6 and TNF-α on HGF secretion were noted in other experimental systems (15, 30). This observation agrees with the fact that HGF promoter region contains a known stimulant of HGF synthesis and secretion in fibroblasts originating from different anatomical locations (9, 24, 34). It is therefore not surprising that, also in our experimental system, inhibition of PGE2 synthesis by indomethacin had suppressive effect on HGF secretion and epithelial cell proliferation in BFE and BE cocultures. PGE2 can be produced by a variety of cell types including epithelial cells and fibroblasts and serve as autocrine and paracrine mediators to signal changes within their immediate environment, suggesting that PGE2 may mediate interactions between BEC and BF through both paracrine and autocrine mechanisms. PGE2 derived from both fibroblast and epithelial compartments may stimulate stromal cells to release growth factor, which in turn provide suitable environment for bronchial epithelium.

In summary, the present data confirm the concept that fibroblasts are able to stimulate airway epithelial cell growth in addition to their other effects, e.g., secretory responses and cell differentiation. Further studies are warranted to elucidate the potential implication of the findings presented here. Further studies should especially examine the role of HGF in diseases accompanied by altered proliferation of BEC.

REFERENCES


