Hepatocyte growth factor and other fibroblast secretions modulate the phenotype of human bronchial epithelial cells

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Myerburg MM, Latoche JD, McKenna EE, Stabile LP, Siegfried JS, Feghali-Bostwick CA, Pilewski JM. Hepatocyte growth factor and other fibroblast secretions modulate the phenotype of human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 292: L1352–L1360, 2007. First published February 16, 2007; doi:10.1152/ajplung.00328.2006.—The luminal airway surface is lined with epithelial cells that provide a protective barrier from the external environment and clear inhaled pathogens from the lung. To accomplish this important function, human bronchial epithelial (HBE) cells must be able to rapidly regenerate a mucociliary layer of cells following epithelial injury. Whereas epithelial-fibroblast interactions are known to modulate the airway architecture during lung development and repair, little is known about how these two cells interact. Using a primary HBE and lung fibroblast coculture system, we demonstrate that 1) subepithelial fibroblasts provide a suitable environment for differentiation of HBE cells into a polarized ciliated phenotype despite being cultured in media that induces terminal squamous differentiation and growth arrest in the absence of fibroblasts, 2) HBE cells cocultured with subepithelial fibroblasts exhibit augmented ciliogenesis, accelerated wound repair, and diminished polarized ion transport compared with cells grown in control conditions, and 3) hepatocyte growth factor (HGF) is important for subepithelial fibroblast modulation of HBE cell differentiation. These results provide a model to study fibroblast modulation of epithelial phenotype and indicate that HGF secreted by subepithelial fibroblasts contributes to HBE cell differentiation.

airway epithelium; ion transport; epithelial-mesenchymal interactions; ciliogenesis

THE EPITHELIAL SURFACE LINING the conducting airways provides a protective barrier from the external environment and clears inhaled and aspirated pathogens from the lung (19). For efficient mucociliary clearance, the bronchial epithelium maintains several properties. First, the apical surface of human bronchial epithelium (HBE) must contain abundant properly functioning cilia that serve to propel overlying mucus from the distal airways to the proximal airways where the mucus can be expelled (51). Second, as the airways are constantly injured by inhaled pathogens, the lining epithelium must be able to rapidly regenerate so that the protective surface is not compromised. Additionally, HBE must be able to properly transport sodium, through the epithelial sodium channel (ENaC), and chloride, through the cystic fibrosis transmembrane conductance regulator (CFTR) and other chloride channels, to maintain the airway surface liquid (ASL) volume required for mucus clearance (28–30). The signaling mechanisms that serve to achieve these phenotypic properties in the epithelial lining surfaces of the airway are not fully understood.

During lung development and during epithelial repair following injury, interactions with subepithelial mesenchymal cells are fundamental to HBE cell migration, proliferation, and differentiation, which are required cellular processes for epithelial restoration (10). During lung development, bidirectional communication within the “epithelial-mesenchymal tropic unit” is necessary for normal bronchial branching and subsequent epithelial differentiation (11). In the developed lung, these epithelial-mesenchymal interactions are important for epithelial repair following injury (18). Known mediators involved in epithelial-mesenchymal interactions include hepatocyte growth factor (HGF), IGF-1, basic fibroblast growth factor, platelet-derived growth factor, endothelin-1, erbB ligands, and transforming growth factor-β (TGF-β), as well as extracellular matrix (ECM) proteins (6, 11, 18, 21, 22, 31, 34, 38, 42, 47, 48, 58, 59). Furthermore, subepithelial fibroblast hyperplasia is a common feature of chronic airway diseases (2, 18, 39) such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis (CF), suggesting that alterations in epithelial-mesenchymal interactions contribute to airway disease pathogenesis. Despite the known importance of the subepithelial mesenchyme in epithelial repair and differentiation, little is known how the two cell types communicate.

The goal of HBE cell culture is to recapitulate native airway biology as much as possible so that investigation can be conducted ex vivo in a simplified environment. Initial attempts to establish well-differentiated HBE cultures were hindered by the use of serum-containing differentiation media, which contains TGF-β that induces terminal squamous differentiation and growth arrest in HBE cells (9, 13, 15, 24, 27). Subsequently, serum-free alternatives were developed that allow for the differentiation of well-polarized, ciliated HBE cell cultures. The most commonly used serum-free differentiation media are 1) bovine pituitary extract-containing media, which is supplemented with various growth factors (see Table 1; Refs. 7, 8, 12, 23, 30, 41, 56), and 2) media containing the proprietary Ultroser G (USG), which is derived in part from calf brain (5, 41, 57). Regardless of the differentiation media used, the ECM composition underlying HBE cells is also important in normal differentiation (4, 37). When cultured in a conductive media at air-liquid interface, HBE cells develop into a pseudostratified polarized layer with apical cilia and basolateral nuclei, which morphologically resembles native airway epithelium. Furthermore, cultured HBE cells exhibit ion transport properties,
HGF DERIVED FROM SUBEPITHELIAL FIBROBLASTS PROMOTES HBE CELL DIFFERENTIATION

L1353

Table 1. Essential supplements in serum-free HBE differentiation media

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Desired Property</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Growth factor</td>
<td>5–10 ng/ml</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Growth factor</td>
<td>0.5–25 ng/ml</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>Mucociliary diff.</td>
<td>0.3–50 nM</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>Mucociliary diff.</td>
<td>−10 μM</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>Augments Na+ absorption</td>
<td>0.2–1.4 μM</td>
</tr>
</tbody>
</table>

HBE, human bronchial epithelium.

specifically sodium and chloride conductances characteristic of ENaC and CFTR, that approximate that of explanted native lung (20).

In an effort to recapitulate the native airway architecture in vitro, we developed an HBE cell and lung fibroblast coculture system utilizing primary human cells derived from excess pathological lung tissue. Similar systems have been previously reported as effective methods of culturing bronchial and skin epithelial cells (25, 48, 53). In the model recently reported by Wiszniewski et al. (53) and Vermeer et al. (48), a subepithelial fibroblast feeding layer in conjunction with HBE differentiation media augmented HBE cell proliferation, increased Na+ transport, and allowed for normal ciliogenesis and mucin production. We further explored the effects of subepithelial fibroblasts on epithelial phenotype and wound repair and assessed the importance of HGF for fibroblast modulation of epithelial differentiation. We provide evidence that 1) subepithelial fibroblasts provide a suitable environment for differentiation of HBE cells into a polarized ciliated phenotype despite being cultured in media that induces terminal squamous differentiation and growth arrest in the absence of fibroblasts, 2) HBE cocultured with subepithelial fibroblasts exhibit augmented ciliogenesis, accelerated wound repair, and diminished polarized ion transport compared with cells grown in control conditions, and 3) HGF is important for subepithelial fibroblast modulation of HBE cell differentiation.

MATERIALS AND METHODS

Materials. All cell culture medium was obtained from Gibco (Invitrogen, Carlsbad, CA) except bronchial epithelial growth media (Clonetics, San Diego, CA) and USG (BioSepra, Cedex, France). Neutralizing HGF antibodies were kindly gifted from Galaxy Biotech (Mountain View, CA), specifically, mouse monoclonal antibody L2G7 to human HGF- and isotype-matched control antibody (mouse IgG3) (17). Recombinant human HGF (rhHGF) was obtained from R&D Systems (Minneapolis, MN). Unless otherwise specified, all other reagents were obtained from Sigma.

HBE and lung fibroblast isolation and culture. HBE cells were cultured from explanted peripheral lung. The tissue was minced, and the fibroblasts were isolated from patients with COPD, idiopathic pulmonary fibrosis, primary pulmonary hypertension, and CF (genotype ΔF508/ΔF508). Qualitative differences in epithelial morphology due to disease state were not observed.

HBE differentiation conditions. HBE-fibroblast cocultures were established by seeding ~5,000 fibroblasts on the undersurface of an inverted Transwell insert containing a confluent layer of undifferentiated HBE on the apical surface. After the fibroblasts had adhered to the filter for 30 min, the Transwell insert was placed into DMEM supplemented with 10% FBS and allowed to differentiate at an air-liquid interface. Alternatively, cells were incubated in fibroblast-conditioned media (FCM) that was established by seeding 5,000 fibroblasts in the bottom of the Transwell in DMEM/10% FBS. For control conditions, HBE cells without subepithelial fibroblasts were allowed to differentiate at air-liquid interface with DMEM/F-12 supplemented with 2% USG as the basolateral media. Epithelial-fibroblast cocultures were also established using HBE cells that had been differentiated in USG-containing media, and experiments were conducted following the indicated number of days in coculture. Schematics of the different culture conditions are provided in the Fig. 1 insets.

In all culture conditions, the basolateral media was changed twice weekly. The rate of media discoloration was similar between control and coculture conditions. In coculture conditions, the subepithelial fibroblasts became confluent after ~1 wk of coculture and a portion of nonadherent fibroblasts grew on the bottom of the well.

Immunohistochemistry. The Transwell inserts were fixed in 4% paraformaldehyde, permeabilized with Triton X-100, and blocked with 5% goat serum prior to incubation with primary antibodies (Sigma-Aldrich, St. Louis, MO). Species-appropriate secondary antibody conjugated to Alexa 488 (Jackson Research, West Grove, PA) was used to visualize tubulin. Additionally, DRAQ5 (Alexis, Lausen, Switzerland) and fluorescently labeled phalloidin (Molecular Probes, Carlsbad, CA) were used as nuclear and cytoskeletal markers, respectively. Images were taken using an Olympus Fluoview 500 confocal microscope and analyzed using a MetaMorph image analysis workstation (Universal Imaging, West Chester, PA). To visualize the epithelial and fibroblast layers independently, the confocal stack was divided at the level of the filter, and each stack was reconstructed separately. To measure the percentage of ciliated cells in the epithelial cell layer, the number of ciliated cells was determined by manual counting random fields and dividing by the total cell count as measured in MetaMorph using automated nuclear counting. In addition to assuring that the investigator was blinded to the conditions, the percentage of pixels occupied by the Alexa 488 was measured in MetaMorph in the same random fields as a complementary method.

Epithelial injury. Mechanical scrape injury was induced by creating a line with a pipette tip across the surface of a confluent HBE cell layer. Following injury, the apical surface was washed with DMEM to remove denuded epithelial cells, and the area of injury was examined to assure uniformity. The HBE cells were serially fixed over 72 h following injury and visualized using F-actin (Molecular Probes) immunohistochemistry. Additionally, the rate of wound closure was assessed by serial measurements of transepithelial resistance (R2TR) using a Millicell electrical resistance system (Millipore, Bedford, MA).
Before $R_{TE}$ measurements, the apical surface of the HBE was submerged in DMEM/F-12 to create an electrical circuit.

**Short-circuit recordings.** Short-circuit currents ($I_{sc}$) were measured as previously described (3, 5, 32). In brief, cells cultured on filter supports were mounted in modified Ussing chambers, and the cultures were continuously short-circuited with an automatic voltage clamp (Department of Bioengineering, University of Iowa, Iowa City, IA). $R_{TE}$ was measured by periodically applying a 2.5-mV bipolar pulse and was calculated using Ohm’s law. The bathing Ringer solution was composed of 120 mM NaCl, 25 mM NaHCO₃, 3.3 mM KH₂PO₄, 0.8 mM K₂HPO₄, 1.2 mM MgCl₂, 1.2 mM CaCl₂, and 10 mM glucose. Chambers were constantly gassed with a mixture of 95% O₂, 5% CO₂ at 37°C, which maintained the pH at 7.4. Following a 5-min equilibration period, the baseline $I_{sc}$ was recorded. To determine the amiloride-sensitive $I_{sc}$, the sodium channel blocker amiloride was added to the apical cell chamber to a concentration of 10 μM.
Subsequently, the cAMP agonist forskolin (10 μM) and then the CFTR channel blocker CFTRinh-172 (10 μM; Calbiochem, San Diego, CA; Ref. 26) were added to determine stimulated CFTR currents.

**Quantitative HGF concentration measurement.** The basolateral media from control, epithelial-fibroblast coculture, and FCM conditions were pooled over a 1 wk interval. This media was concentrated by centrifugal ultrafiltration with a Microcon YM-10 concentrator. HGF concentration was determined using the Quantikine human HGF immunoassay (R&D Systems) per the manufacturer’s instructions.

**Assessment of the effects of HGF on HBE cell phenotype.** To assess the contribution of HGF to HBE cell phenotype in control and fibroblast coculture conditions, HBE cells were cultured in the presence and absence of 300 ng/ml neutralizing HGF antibody L2G7 or 10 ng/ml rhHGF for 21 days. In control conditions, isotype-matched mouse IgG2a was used at the same concentration. All antibodies and rhHGF were added to the basolateral media, which was changed twice weekly. Following 21 days of incubation, the R<sub>TE</sub>, cell density, and percentage of ciliated cells were measured as described above.

**Statistics.** Results are expressed as means ± SE. Significance was determined by ANOVA with Bonferroni post hoc analysis or by Student’s t-test where appropriate, unless otherwise indicated. Because of inherent variability between donors, the data shown are the results obtained from one donor, unless otherwise indicated. Similar results were observed in independent experiments using three different donors.

**RESULTS**

**Epithelial-fibroblast interactions promote normal epithelial differentiation.** To determine whether factors secreted by subepithelial fibroblasts are sufficient to promote normal HBE differentiation, we compared the morphology of HBE cells cultured in serum-free differentiation media (2% USG) to that of HBE cocultured with subepithelial fibroblasts in 10% FBS. As previously shown (8, 9), HBE cells cultured at an air-liquid interface in USG developed into a pseudostratified columnar epithelium, with apical cilia and nuclei organized towards the basolateral surface (Fig. 1A). When the USG was replaced with 10% FBS, the HBE cells developed into a stratified squamous layer with a decreased cell density (data not shown), presumably as a result of the TGF-β present in the serum (9, 13, 15, 24, 27). In contrast, when primary lung fibroblasts were cocultured on the undersurface of the Transwell filter, HBE cells developed into a normal polarized ciliated layer, despite the presence of serum (Fig. 1C). This suggests that subepithelial fibroblasts secrete factors that promote normal epithelial differentiation. To determine whether the epithelial-mesenchymal interaction requires that the cells are in near proximity, we cultured HBE cells in FCM, which was established by culturing fibroblasts on the bottom of the Transwell in 10% FBS. Under these conditions, in which the subepithelial mesenchyme was not adjacent to the HBE, the epithelial layer developed into a stratified squamous epithelium (Fig. 1B), similar to the phenotype seen when the HBE cells were cultured in FBS alone. These results suggest that direct epithelial-mesenchymal interactions are important for normal HBE cell differentiation.

**Epithelial-fibroblast interactions promote ciliogenesis.** To further characterize the effect of the subepithelial fibroblasts on HBE cell differentiation, we examined the number of ciliated cells present in epithelial-fibroblast coculture compared with standard culture (Fig. 2, A and B). HBE cells that were cocultured for 2 wk developed 16.8 ± 3.5% (n = 4; P = 0.003) more ciliated cells as measured by manual counting of...

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**Fig. 2.** Effect of epithelial-fibroblast interactions on ciliogenesis after 2 wk in culture. Shown are confocal microscopy images of cilia in control (A) vs. coculture (B) conditions using immunocytochemistry for acetylated tubulin as a ciliary marker identified with Alexa 488 (green) (magnification ×400). C: mean percent cilia in control and coculture HBE cells. Cilia were quantified by dividing the number of ciliated cells by the total cell count (%Cells, black bars) and by the percentage of pixels occupied by Alexa 488 (%Area, gray bars). Data shown are mean percentages of cilia ± SE, n = 4 filters. *Significantly different from control conditions.
random tubulin-labeled fields (Fig. 2C); this represents a 40% increase in ciliated cells compared with standard USG culture methods. As an alternate measurement of the amount of cilia, these results were confirmed by measurement of the total fluorescence generated by the cilia marker acetylated tubulin. Similar to the results obtained by manual counting, there was a $12.9 \pm 3.8\%$ ($P = 0.015$) increase in the area covered by cilia as measured by image analysis of random fields (Fig. 2D). These results suggest that factors secreted by subepithelial fibroblasts either promote ciliogenesis or prolong ciliated cell survival.

**Epithelial-fibroblast interactions accelerate epithelial wound repair.** Because factors secreted by fibroblasts are known to accelerate epithelial wound repair (18), we next investigated whether epithelial-fibroblast coculture would affect HBE wound closure. Matched HBE cell cultures that were initially differentiated in USG were grown for 2 wk in 2% USG, FCM, or epithelial-fibroblast coculture. Subsequently, the epithelial layer was mechanically injured, the filters were serially fixed, and F-actin immunohistochemistry performed. Twenty hours following epithelial denudation, the scrape injury remained visibly patent in the control conditions (Fig. 3A) and was partially closed in FCM conditions (Fig. 3B). However, in the HBE cells that were cocultured with fibroblasts, the wound was completely reepithelialized at the same time point (Fig. 3C). As an additional measure of the rate of epithelial repair, wound closure was assessed by serial measurements of $R_{TE}$. As expected, $R_{TE}$ sharply declined following epithelial injury and subsequently increased as the wound closed to levels exceeding baseline (Fig. 3D). The rate of restoration of $R_{TE}$ was markedly accelerated in epithelial-fibroblast cocultures compared with control ($n = 4; P < 0.001$; Fig. 3D). The rate of epithelial wound closure in FCM was accelerated with respect to control conditions, although not to the degree seen in fibroblast coculture, suggesting that ECM and secreted factors from the subepithelial mesenchyme are important for epithelial regeneration following injury.

**HBE cells cultured in epithelial-fibroblast coculture exhibit diminished polarized ion transport.** Based on our findings that fibroblast coculture augmented ciliogenesis and accelerated wound closure, we anticipated that the electrophysiological properties of HBE cells would also be heightened. To test this, HBE cultures were placed in Ussing chambers following 21 days of culture in 2% USG, FCM, or epithelial-fibroblast coculture. As shown in Fig. 4, HBE cultured in 2% USG generated a standard $I_{sc}$ tracing for normal HBE cells (Fig. 4A, top tracing). A large amiloride-sensitive (ENaC) current comprised the majority of the transepithelial current, and a smaller

![Fig. 3.](http://ajplung.physiology.org/)
forskolin-stimulated CFTR current was also present. However, when cultured in FCM or in epithelial-fibroblast coculture, the magnitude of the transepithelial current was markedly diminished. Summary data derived from four independent experiments (3 non-CF HBE cell cultures and 1 CF culture) are shown in Table 2. These findings indicate that the morphological and electrophysiological phenotypes of HBE cells are dictated by different mechanisms and suggest that factors other than the underlying mesenchyme are important in regulating the ion transport properties of airway epithelium.

Next, we tested whether additives to the basolateral media could increase polarized current in the coculture system. In standard HBE cell differentiation media, hydrocortisone is typically added to serum-free HBE differentiation media to augment ENaC current (41). Therefore, we added 0.1 μM hydrocortisone to the basolateral media (Fig. 4B). This had no effect on the PD on the control conditions ($P = 0.19$; $n = 6$), suggesting that there is sufficient glucocorticoid in USG to stimulate ENaC. However, hydrocortisone more than doubled the PD in the cocultures ($P = 0.0001$; $n = 6$). Therefore, the reduced polarized ion transport in this epithelial-fibroblast coculture system can be augmented with the addition of physiological regulators of ENaC activity.

**HGF from subepithelial fibroblasts contributes to HBE cell differentiation.** Because HGF is secreted from fibroblasts (33) and the HGF receptor (c-Met) is expressed on the basolateral surface of HBE (42), we reasoned that HGF secreted by subepithelial fibroblasts may be a critical growth factor to support normal HBE differentiation. Furthermore, HGF has been shown to increase ciliogenesis (42), decrease the amiloride-sensitive $I_{sc}$ (43), and counteract the effects of TGF-β (46) in a manner similar to what was observed in epithelial-fibroblast coculture. To determine the amount of HGF secreted by subepithelial fibroblasts, ELISA was performed on the basolateral media from control, coculture, and FCM. As shown in Fig. 5A, HGF is present in the fibroblast containing cultures in sufficient concentrations to act as a mitogen and stimulate ciliogenesis in HBE (33, 34, 42). In control conditions and media alone, minimal amounts of HGF were present. These results confirm that subepithelial fibroblasts secrete HGF in sufficient concentrations to support epithelial differentiation.

To assess the contribution of HGF derived from the subepithelial mesenchyme on HBE cell phenotype, we treated control and epithelial-fibroblast cultures with an HGF-neutralizing antibody or an isotype-matched control antibody for 21 days prior to measurement of $R_{TE}$, cell density, and cilia density (Fig. 5, B–D). Neutralization of HGF with 300 ng/ml neutralizing antibody L2G7 markedly decreased the $R_{TE}$, cell density, and percentage of ciliated cells in coculture conditions. However, HGF neutralization had no effect on the HBE cell phenotype when the cells were cultured in USG. These results indicate that HGF derived from the subepithelial fibroblasts is a critical factor in the development of a normal HBE phenotype in serum-containing media. Next, as shown in Fig. 5, B–D, we assessed the effects of recombinant HGF on HBE cells cultured in control and epithelial-fibroblast coculture conditions. Whereas 10 ng/ml rhHGF did not affect the $R_{TE}$, rhHGF increased the cell density and amount of cilia present in both control and coculture conditions. These results are in agreement with previous reports that HGF is important in ciliogenesis and acts as a mitogen for HBE (33, 34, 42). Thus subepithelial mesenchymal cells elaborate HGF, which is an important mediator for normal epithelial differentiation in our coculture system.

**Table 2. Electrophysiological effects of epithelial-fibroblast coculture**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Coculture</th>
<th>% Change</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{TE}$ (Ω cm$^2$)</td>
<td>547.6 ± 31.4</td>
<td>537.6 ± 37.3</td>
<td>1.9</td>
<td>0.82</td>
</tr>
<tr>
<td>PD (mV)</td>
<td>10.3 ± 1.48</td>
<td>4.1 ± 1.12</td>
<td>60.2</td>
<td>0.01</td>
</tr>
<tr>
<td>$I_{sc}$ (μA/cm$^2$)</td>
<td>26.1 ± 2.7</td>
<td>9.3 ± 2.48</td>
<td>64.2</td>
<td>0.0003</td>
</tr>
<tr>
<td>ENaC current (μA/cm$^2$)</td>
<td>13.6 ± 1.06</td>
<td>3.2 ± 1.27</td>
<td>76.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>CFTR current (μA/cm$^2$)</td>
<td>5.2 ± 0.29</td>
<td>3.1 ± 0.51</td>
<td>40.4</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values are means ± SE, $n > 2$ filters from 3 normal and 1 cystic fibrosis donors. PD and CFTR current is from normal donors only. $R_{BE}$, transepithelial resistance; $I_{sc}$, short circuit current; ENaC, epithelial sodium channel; PD, potential difference.
DISCUSSION

Despite the importance of epithelial-mesenchymal interactions in lung development and epithelial repair, little is known about how epithelial cells and fibroblasts interact in vitro. Using primary cultures of HBE cells and human lung fibroblasts, we demonstrate that 1) subepithelial fibroblasts provide a suitable environment for differentiation of HBE cells into a polarized ciliated phenotype, despite being cultured in media that induces terminal squamous differentiation and growth arrest in the absence of fibroblasts, 2) HBE cells cocultured with subepithelial fibroblasts exhibit augmented ciliogenesis, accelerated wound repair, and diminished polarized ion transport compared with cells grown in control conditions, and 3) HGF is involved in subepithelial fibroblast modulation of HBE cell differentiation. These results indicate that the local environment established by subepithelial mesenchymal cells promotes HBE cellular differentiation and augments epithelial restoration following injury in the airways.

Although the HGF concentration was similar in coculture and FCM conditions, the subepithelial fibroblasts were only capable of promoting a well-differentiated HBE layer when the two cell types were in close proximity. We hypothesize that the local concentration of HGF and potentially other growth factors is increased when the subepithelial fibroblasts are in close proximity to the overlying HBE. Other mediators with shorter half-lives may also be secreted by the epithelium and fibroblasts and have trivial concentrations in the FCM conditions. Alternatively, the subepithelial fibroblasts may provide a barrier that isolates the HBE from the effects of TGF-β and other serum components to the epithelial cells. Further studies of the coculture matrix will likely be informative.

Unexpectedly, whereas epithelial-fibroblast coculture promoted normal epithelial morphological differentiation, we found that HBE cells cultured in this model developed minimal polarized ion transport. Indeed, these electrophysiological properties can be partially restored in coculture conditions with the addition of hydrocortisone (Fig. 4B) or in the presence of USG (53). Because cultured HBE cells are able to regulate ASL volume, it is believed that regulation of ion transport is dictated by autocrine mechanisms inherent to HBE cells. Since epithelial-fibroblast coculture was sufficient to promote a morphologically normal epithelial layer, the paucity of vectorial ion transport in coculture suggests that alternative mechanisms exist in vivo to regulate ion transport. We speculate that these nonautocrine factors are important for ENaC and CFTR channel expression in HBE cells but may not necessarily be involved in the physiological regulation of the activity of these channels.

There has recently been a resurgence of investigation into the plasticity of airway epithelium. In vivo, basal bronchial epithelial cells are capable of assuming ciliated, squamous, columnar, and goblet cell phenotypes following injury (1). In a recent murine model reported by Tyner et al. (45), the development of epithelial hyperplasia and mucus metaplasia in the airway involved transdifferentiation of ciliated cells to goblet cells. Additionally, epithelial-to-mesenchymal transition has been demonstrated in lung epithelium, at least in animal
models (14, 52, 54, 55), and is believed to be involved in the pathogenesis of lung cancer (16), obliterative bronchiolitis (49), and idiopathic pulmonary fibrosis (52). Our findings provide additional laboratory evidence of epithelial cell plasticity, and demonstrate that the morphological and electrophysiological phenotypes of HBE cells are not intimately linked. Airway cell differentiation into a ciliated epithelium may be necessary for normal mucociliary clearance, however, ciliary differentiation is not sufficient for establishment of significant chloride and sodium conductances. Hence the ion transport properties of airway epithelium appear regulated by additional mechanisms.

In summary, these data suggest that secretion of HGF from subepithelial mesenchymal cells is important for the preservation of epithelial integrity and mucociliary clearance in the airways. Traditionally, subepithelial fibroblast hyperplasia is regarded as a pathological feature of airway remodeling and believed to contribute to airway luminal narrowing in obstructive airway diseases. Furthermore, heightened levels of HGF are known to promote bronchial neoplasia (35, 40, 44, 50). Because subepithelial fibroblasts elaborate factors that increase the rate of wound closure and augment ciliogenesis, we speculate that subepithelial fibroblast proliferation following epithelial injury may have a beneficial role in airway epithelial repair.

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