KGF alters gene expression in human airway epithelia: potential regulation of the inflammatory response

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Keratinocyte growth factor (KGF) regulates several functions in adult and developing lung epithelia; it causes proliferation, stimulates secretion of fluid and electrolytes, enhances repair, and may minimize injury. To gain insight into the molecular processes influenced by KGF, we applied KGF to primary cultures of well-differentiated human airway epithelia and used microarray hybridization to assess the abundance of gene transcripts. Of 7,069 genes tested, KGF changed expression levels of 910. Earlier studies showed that KGF causes epithelial proliferation, and as expected, treatment altered expression of numerous genes involved in cell proliferation. We found that KGF stimulated transepithelial Cl\textsuperscript{−} transport, but the number of cystic fibrosis (CF) transmembrane conductance regulator (CFTR) transcripts fell. Although transcripts for ClC-1 and ClC-7 Cl\textsuperscript{−} channels increased, KGF failed to augment transepithelial Cl\textsuperscript{−} transport in CF epithelia, suggesting that KGF-stimulated Cl\textsuperscript{−} transport in differentiated airway epithelia depends on the CFTR Cl\textsuperscript{−} channel. Interestingly, KGF decreased transcripts for many interferon (IFN)-induced genes. IFN causes trafficking of Stat dimers to the nucleus, where they activate transcription of IFN-induced genes. We found that KGF prevented the IFN-stimulated trafficking of Stat1 from the cytosol to the nucleus, suggesting a molecular mechanism for KGF-mediated suppression of the IFN-signaling pathway. These results suggest that in addition to stimulating proliferation and repair of damaged airway epithelia, KGF stimulates Cl\textsuperscript{−} transport and may dampen the response of epithelial cells to inflammatory mediators.

Previous studies indicate that KGF has a variety of pulmonary effects. KGF stimulates epithelial cell proliferation in the airway and alveoli; this occurs in both the developing and adult lung (26, 27, 37, 44). KGF also affects the morphology of the developing lung as evidenced by studies of transgenic mice. Disrupting KGF receptor function by expressing a dominant negative form of the receptor significantly reduced airway branching during development (11, 29). Moreover, over-expressing KGF in the airways of developing mice severely altered lung growth (33). The lungs of these animals closely resembled those observed in congenital cystadenomatous malformation (CCAM), a disease of abnormal human lung development (25).

KGF also stimulates fluid and electrolyte secretion. When applied to fetal lung explants, KGF stimulates dramatic intraluminal swelling and expansion (10). Bumetanide, which blocks the Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransporter, inhibits KGF-induced swelling (15), suggesting that explant swelling is driven by Cl\textsuperscript{−} secretion. KGF may also play a role in repair of the airway following injury. KGF reduced the mortality associated with acute lung injury in animal models (16). As a result, a role for KGF in the treatment of the acute respiratory distress syndrome (ARDS) has been proposed (40). KGF may also prevent airway damage from Pseudomonas aeruginosa infection (38).

Given the potential importance of KGF in epithelial proliferation, lung development, disease, and potentially new therapies, it is important to understand the molecular processes under KGF influence. Moreover, identification of KGF-induced changes in gene expression might suggest novel targets for KGF treatment. To investigate the response of airway epithelia to KGF, we examined the effect of KGF on the expression level of a large number of genes. We used an in vitro model of human airway epithelia; when grown at the air-liquid interface, primary cultures of airway cells differentiate to form a pseudostratified, ciliated epithelium (43, 45). We took advantage of microarray technology to examine the expression level of ~7,000 different human genes using Affymetrix GeneChip microarrays (21). By measuring the expression level of these genes, we hoped to identify groups of genes that may play integral roles in lung development, airway proliferation, and epithelial repair.
METHODS

Cell culture. Human airway epithelia were isolated and cultured as previously described (43, 45). Cells from either trachea or bronchus were seeded onto collagen-coated permeable supports (Millicell; Millipore, Bedford, MA) and grown at the air-liquid interface. Epithelia were studied at least 2 wk after seeding when they had developed a transepithelial resistance and a differentiated function and morphology (45). Epithelia were treated with 50 ng/ml KGF (Amgen, Thousand Oaks, CA) applied to the basolateral media for 0, 8, or 20 h.

Electron microscopy. Epithelia were washed and fixed in 2.5% glutaraldehyde and processed using standard electron microscopic procedures. Samples were postfixed in 1% osmium tetroxide followed by 2.5% aqueous uranyl acetate and then dehydrated in a graded series of ethanol washes. Thin sections (70 nm) of the Eponate 12-embedded specimen were placed on 135-mesh hexagonal copper grids and poststained with uranyl acetate and Reynold’s lead citrate. The height of epithelia was measured from the basolateral filter to the apical membrane (base of the cilia). Nine measurements were made for each epithelium. These were averaged and normalized to the height of control, untreated epithelia. Statistical significance was measured with an unpaired t-test.

Measurement of transepithelial Cl− current. For measurement of transepithelial electrical properties, airway epithelia grown on membrane supports were mounted in modified Ussing chambers (Jim’s Instruments, Iowa City, IA) and studied as previously described (34). Transepithelial voltage was clamped to 0 mV using a dual-voltage clamp (model 6162C-2; Bioengineering, University of Iowa, Iowa City, IA), and short-circuit current (Isc) was recorded continuously. Epithelia were bathed in symmetrical solutions containing (in mM) 135 NaCl, 2.4 K2HPO4, 0.6 KH2PO4, 1.2 CaCl2, 1.2 MgCl2, 10 dextrose, and HEPES with pH adjusted to 7.2 with 10 N NaOH. Solutions were continuously gassed with humidified 100% O2. For studies on non-cystic fibrosis (CF) epithelia, the following agents were sequentially added to the apical surface: amiloride (100 μM), DIDS (100 μM), and the cAMP agonists forskolin (10 μM) and 3-isobutyl-1-methylxantine (IBMX, 100 μM). Bumetanide (100 μM) was then added to the basolateral surface. For studies on CF epithelia, identical solutions were used, with the sequential addition of amiloride, cAMP agonists, ATP (apical, 1 mM), DIDS, and bumetanide. The later addition of DIDS in CF epithelia aimed to detect any of the cAMP- or ATP-stimulated changes in current that might have been DIDS inhibitable.

RNA isolation, target preparation, and microarray analysis. Target preparation was performed according to Affymetrix protocols. Total RNA was isolated from airway epithelia by application of 0.5 ml of RNA Stat-60 (Tel-test, Friendswood, TX) to each epithelium followed by repeated pipetting. Total RNA was purified according to manufacturer’s instructions. DNase I treatment and a subsequent RNeasy (Qiagen, Valencia, CA) purification step were included prior to reverse transcription. Double-stranded cDNA was synthesized from total RNA using SuperScript II (GIBCO-BRL; Life Technologies, Rockville, MD). Fluorescent cRNA was produced by in vitro transcription (Enzo, New York, NY). Following removal of unincorporated dNTPs, cRNA was fragmented by heating to 94°C in the presence of magnesium acetate (30 mM) and potassium acetate (100 mM). Hybridization cocktail containing the fragmented cRNA was incubated overnight with Affymetrix HuFL GeneChips. The GeneChips were processed and scanned on an Affymetrix Fluidics Station and Scanner (Hewlett-Packard, Palo Alto, CA). Gene expression data were produced using GeneChip software. The data were converted into tab-delimited text format for subsequent data analysis.

Data analysis. Expression data were collected in an Excel spreadsheet (Microsoft, Redmond, WA). For expression analysis, the relative fluorescence intensity was calculated by subtracting the fluorescence hybridization intensity of a mismatch control probe from the perfect match probe for each of 20 different probe pairs for each gene. The relative fluorescence intensities for each gene were then averaged at each time point (n = 3 for t = 0 h; n = 2 for t = 8 h and 20 h). After reformatting, self-organized maps were generated using GeneCluster (Whitehead Institute, Massachusetts Institute of Technology) (36). The data set was filtered to include only genes whose expression changed during KGF treatment (maximum expression − minimum expression > 75 units; and maximum/minimum > 2). Genes were only included if their expression level at one time point was greater than 50 units. For cluster analysis of ion channels and transporters, many of which are expressed at very low copy number, the thresholds were lowered to the difference (max − min) > 40 units and the ratio (max/min) > 1.5. Default parameters were used for the remaining settings. The number of clusters within self-organizing maps (SOMs) was adjusted until the error within clusters was minimized without producing redundancy. For normalization of gene expression patterns, the mean expression level for each gene during the experiment was set equal to 0. The normalized expression level for each gene x at time t was defined as: [(expression level of gene x at time t) − (mean expression level of gene x)]/standard deviation of expression level of gene x). In smaller self-organized maps using data for specific groups of genes, the data are shown normalized to the expression level at t = 0 h.

Stat1 immunoblotting. Airway epithelia were washed three times in ice-cold PBS containing 1 mM MgCl2 and 0.1 mM CaCl2. The cells were lysed in Tris-buffered saline (TBS) containing 1% Triton X-100, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 0.4 mM phenylmethylsulfonyl fluoride, and 10 μg/ml pepstatin A. Cells were solubilized at 4°C for 20 min. Cell lysates were centrifuged at 14,000 g for 30 min at 4°C. Following centrifugation, the clear, cytosolic fraction was isolated and transferred to a new, prechilled microcentrifuge tube. After gently removing the mitochondrial fraction, the nuclear pellet was washed with ice-cold PBS, briefly centrifuged, and solubilized in 2% SDS at 95°C for 30 min. One-fourth of each sample was separated by SDS-PAGE using 8% polyacrylamide gels. Following transfer to a polyvinylidene difluoride membrane and blocking with 5% bovine serum albumin and 0.05% Tween-20, Stat1 protein was detected using a monoclonal antibody directed against the carboxy terminus of human Stat1 (Transduction Laboratories, Franklin Lakes, NJ). For interferon (IFN)-treated samples, airway epithelia were simultaneously exposed to 100 U/ml of IFN-γ (Genentech, San Francisco, CA) and 100 U/ml of IFN-α (Schering, Kenilworth, NJ) for 30 min prior to cell lysis.

RESULTS AND DISCUSSION

Effect of KGF on morphology of airway epithelia. Primary cultures of airway epithelia provide a model system for studying multiple aspects of airway biology, including ion transport, gene expression, and structural morphology. We applied KGF (50 ng/ml) to epithelia and examined the morphology using transmission electron microscopy. After treatment for 20 h, the height of the epithelium increased (Fig. 1). The
changes appeared to be diffuse throughout the epithelia; we did not observe focal areas of increased height. These results are consistent with results showing increased bromodeoxyuridine staining throughout cultured airway epithelia following KGF treatment (39) and earlier studies showing proliferation of pulmonary epithelia in various other model systems (26, 27, 37, 44).

Assessment of KGF-induced changes in mRNA. We hypothesized that KGF must regulate the expression of a large number of genes to account for its various functions in airway biology. To test this with a broad, unbiased approach, we used Affymetrix HuFL GeneChips to measure changes in mRNA levels for ~7,000 previously identified human genes. Airway epithelia were treated with 50 ng/ml KGF for 0, 8, or 20 h. Following RNA isolation, cDNA was reverse-transcribed and fluorescent cRNA was produced by in vitro transcription. cRNA was hybridized against the GeneChips, and the relative fluorescence intensity for each gene was detected by laser scanning. The expression data were then analyzed using GeneCluster.

We found that 3,794 genes (out of 7,069 examined) were expressed at levels above the inherent noise of the microarray hybridization for at least one time point in either the control or KGF-treated epithelia (expression data for all 7,069 genes is provided in the Supplementary Material1 to this article, published online at the Physiological Genomics web site). KGF treatment caused a twofold or greater change in the expression level of 910 genes. These 910 genes were clustered into a SOM, which grouped genes into a predetermined number of clusters based upon their pattern of expression (36). Grouping the KGF-affected genes into eight separate clusters yielded a reasonable separation between clusters without redundancy. Figure 2 shows the multiple patterns of gene expression. Expression of some genes increased or decreased 8 h after KGF treatment but returned closer to baseline levels by 20 h (see clusters 3 and 7). Expression of other genes increased or decreased only following 20 h of KGF exposure. A few of the clusters represent divisions within a continuum of expression patterns. For example, genes within clusters 2 and 3 (Fig. 2) both increased at 8 h of KGF treatment and then returned close to baseline at 20 h. If the 20 h level was above baseline, the gene was grouped into cluster 3. If lower than baseline, then the gene was grouped into cluster 2.

From individual genes showing altered expression, we identified groups of genes involved in common cellular processes. For example, many of the genes showing altered transcript levels are associated with cell growth (Table 1). We found decreased expression of several tumor suppressor genes and other genes that act as negative regulators of cell division. These results are consistent with the known role of KGF in stimulating epithelial proliferation (41). Interestingly, ex-
pression of the KGF receptor, FGFR2, decreased following KGF treatment, suggesting negative feedback regulation.

**Effect of KGF on electrolyte transport by airway epithelia.** As newly formed lung buds migrate through the mesenchyme, they expand by secreting fluid and electrolytes into the lumen (5, 23). KGF, together with FGF-10, is critical to this process (3, 15, 30). To learn whether KGF alters electrolyte transport, we treated epithelia with KGF, and measured $I_{sc}$ 20 h later. Basal $I_{sc}$ and the amount of amiloride-inhibited current were the same in control and KGF-treated epithelia (Fig. 3). There was little if any DIDS-sensitive current in either control or KGF-treated epithelia. However, cAMP agonists stimulated current to a greater extent in epithelia treated with KGF (Fig. 3). Moreover, the bumetanide-inhibited current was twice as large in the KGF-treated epithelia (25.9 ± 2.1 vs. 12.6 ± 1.1 μA/cm², $P < 0.001$). These results suggest that KGF enhanced the CF transmembrane conductance regulator (CFTR)-dependent transepithelial Cl⁻ transport pathway. The underlying mechanism might involve KGF-induced alteration in the production, regulation, or posttranslational modification of the transporters or regulatory molecules involved.

Because KGF stimulated Cl⁻ transport, we examined the effect on genes encoding ion transporters and channels (Fig. 4). Although 20 h exposure to KGF increased cAMP-stimulated current (Fig. 3), CFTR mRNA levels decreased after 20 h of KGF treatment. KGF also elevated the number of transcripts of two other Cl⁻ channels, CIC-1 and CIC-7. However, it is possible that the levels of mRNA may not correlate with protein levels and hence physio-

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**Fig. 2.** Self-organizing map (SOM) of genes affected by KGF treatment. Differentiated airway epithelia were treated with KGF (50 ng/ml) for 0, 8, or 20 h. Following data filtering to remove genes whose expression level did not change with KGF, expression data for 910 genes were clustered into SOMs. Normalized expression levels were calculated using the following formula: $(\text{expression level of gene } x \text{ at time } t) - (\text{mean expression level of gene } x) / (\text{standard deviation of expression level of gene } x)$. 

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
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<tbody>
<tr>
<td>1</td>
<td>238</td>
</tr>
<tr>
<td>2</td>
<td>182</td>
</tr>
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<td>7</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
</tr>
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</table>
Table 1. KGF regulates the transcription levels of genes implicated in cell growth and proliferation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 h</td>
<td>20 h</td>
</tr>
<tr>
<td>Leupin</td>
<td>+2.6</td>
<td>Protease inhibition/leaves apoptosis</td>
</tr>
<tr>
<td>IPL</td>
<td>+4.5</td>
<td>Gene imprinting</td>
</tr>
<tr>
<td>FGF-bp</td>
<td>+3.3</td>
<td>Retinoid-sensitive FGF binding protein</td>
</tr>
<tr>
<td>PCNA</td>
<td>+2.0</td>
<td>DNA replication and repair</td>
</tr>
<tr>
<td>P1-ecd47</td>
<td>+1.6</td>
<td>DNA replication</td>
</tr>
<tr>
<td>GGF2</td>
<td>+1.5</td>
<td>Ligand for heregulin receptor HER-3</td>
</tr>
<tr>
<td>Emp-3</td>
<td>+2.1</td>
<td>Expressed in some tumors</td>
</tr>
</tbody>
</table>

Decreased

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF2</td>
<td>−1.3</td>
<td>KGF receptor</td>
</tr>
<tr>
<td>FGF3</td>
<td>−1.7</td>
<td>FGF receptor</td>
</tr>
<tr>
<td>HFH-3</td>
<td>−1.6</td>
<td>Cell fate determining</td>
</tr>
<tr>
<td>TTF-1</td>
<td>−3.5</td>
<td>Cell fate determining</td>
</tr>
<tr>
<td>Gas1</td>
<td>−1.8</td>
<td>Growth arrest</td>
</tr>
<tr>
<td>Gas6</td>
<td>NC</td>
<td>Growth arrest</td>
</tr>
<tr>
<td>TS2</td>
<td>−1.3</td>
<td>Presumed tumor suppressor (mutated in tuberous sclerosis)</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>−1.2</td>
<td>IGF binding protein</td>
</tr>
<tr>
<td>CS1</td>
<td>−1.5</td>
<td>Checkpoint suppressor</td>
</tr>
<tr>
<td>Cyclin G2</td>
<td>NC</td>
<td>Regulator of cell cycle</td>
</tr>
<tr>
<td>B-cell TG1</td>
<td>−1.1</td>
<td>Anti-proliferative</td>
</tr>
</tbody>
</table>

Following cluster analysis shown in Fig. 3, members of various clusters with presumed function related to cell growth or proliferation were grouped into genes whose expression level increased or decreased with KGF treatment. Fold change was calculated as follows. Increased expression: [(expression level for gene x at time t) − (expression level for gene x prior to KGF exposure)]/expression level for gene x prior to KGF exposure + 1. Decreased expression: [(expression level for gene x at time t) − (expression level for gene x prior to KGF exposure)]/expression level for gene x prior to KGF exposure − 1. KGF, keratinocyte growth factor; FGFR, fibroblast growth factor; and IGFBP, insulin-like growth factor; PCNA, proliferating cell nuclear antigen; NC, no change; IPL, imprinted in placenta and liver; GGF, glial growth factor; HFH, hepatocyte nuclear factor-3/forkhead homolog; TTF, thyroid transcription factor.

logical function. Thus we further assessed function as described below.

Previous studies have shown that CIC-1 is highly expressed in skeletal muscle but also to a lesser extent in other organs (35). Mutations in CIC-1 cause defective repolarization of the muscle membrane voltage, resulting in congenital myotonia (19). The function of CIC-1 in nonmuscle membranes is unknown but may include regulation of plasma membrane resting potential (17). CIC-7 is expressed ubiquitously and may play a role in endosomal acidification (20). The HuFL microarray used does not include probes specific for CIC-2. However, KGF has been reported to produce a 20% increase in CIC-2 protein in fetal rat distal lung epithelial cells (4). The significance of this observation for pulmonary electrolyte transport is uncertain, because CIC-2 deficient mice lack overt pulmonary abnormalities (8). Studies in fetal mouse lung also suggest that Cl− channels other than CFTR may be involved in transepithelial Cl− transport. KGF stimulated the expansion of fetal lung explants from both wild-type and CFTR null mice (46), and the β-adrenergic agonist terbutaline increased the luminal voltage in tracheal explants from both wild-type and CFTR null mice (2).

The findings that KGF treatment increased Cl− current and the number of CIC-1 and CIC-7 transcripts but not the number of CFTR transcripts suggested that KGF might stimulate a pathway for transepithelial Cl− current independent of CFTR. If such a pathway were to exist, then we hypothesized that it would be present in epithelia lacking functional CFTR, i.e., in CF epithelia. We tested this hypothesis by treating CF epithelia with KGF and measuring Isc. Figure 5 shows that KGF exposure decreased basal and amiloride-inhibited Isc. However, it increased neither cAMP-stimulated current nor ATP-stimulated current. Moreover, the responses to DIDS, which inhibits several members of the CIC family (18, 32), and bumetanide were not altered by KGF. We cannot exclude the possibility that a non-CFTR, DIDS-insensitive pathway for transepithelial Cl− transport exists in non-CF epithelia. However, these results suggest that, at least in this differentiated model of human airway epithelia, KGF stimulated a pathway for transepithelial Cl− transport that involves CFTR.

Of the three epithelial Na+ channel (ENaC) subunits, α, β, and γENaC, only the expression of γENaC increased with KGF exposure (Fig. 4). Yet KGF did not alter the amiloride-sensitive, transepithelial Na+ current in non-CF airway epithelia (Fig. 3). Because optimal ENaC function requires the expression of all three subunits (9, 24), the lack of effect on current is perhaps not surprising. Nevertheless, the KGF-induced increase in γENaC mRNA levels, as well as the reduced amiloride-sensitive basal current in CF epithelia may warrant further investigation.

For liquid to follow salt transport, the epithelium must be water permeable. Thus it is interesting that...
KGF increased the mRNA for aquaporins 3 (AQP3) and 5 (AQP5) (Fig. 4). These proteins form water channels in the cell membrane. AQP5 is expressed on the apical membrane of type I alveolar epithelial cells and may contribute to fluid movement into and out of alveoli. Treatment of type II alveolar cells with KGF promotes the conversion to a more type I appearance and increases the expression of AQP5 (7). AQP3 is also present in basal cells of the airway and in the basolateral membranes of acinar cells within secretory glands. AQP3 is permeable to both water and glycerol, but its role in the maintenance of airway liquid is unknown (6). AQP2, AQP6, and AQP7 were also expressed in human airway epithelia, but KGF treatment did not alter their expression levels (see Supplemental Materials).

KGF alteration of IFN-induced Stat1 translocation. Based on the effect of KGF on cell proliferation and fluid and electrolyte transport, we had expected to find changes in genes involved with these processes. However, we were surprised to find alterations in a number of genes involved in the cellular response to inflammation. Interestingly, the expression of many IFN-induced genes decreased following KGF treatment. Cluster analysis of IFN-associated genes produced three groups (Fig. 6). After 8 h of exposure to KGF, transcripts of many genes had decreased, although a few increased and some did not change. However, by 20 h after KGF addition, 22 of the 23 genes that changed with KGF treatment showed decreased numbers of transcripts.

To investigate further the KGF-induced reduction of IFN-related genes, we examined the response to IFN. IFNs bind receptor complexes on the cell surface. Ligand binding to the receptor activates a tyrosine phosphorylation cascade. Stat molecules are targets of this cascade, and once phosphorylated, cytosolic Stat proteins dimerize and traffic to the nucleus. Following nuclear import, Stat dimers complex with other proteins and activate IFN-induced genes. Our observation that KGF decreases the expression of multiple IFN-induced genes suggested interference at some point along this pathway. We hypothesized that KGF prevented the IFN-induced trafficking of Stat molecules from the cytosol to the nucleus.

To test this hypothesis, we treated epithelia with KGF for 20 h and then stimulated them with IFN. Following 30 min of IFN exposure, we lysed the cells and isolated cytosolic and nuclear fractions. Stat1 was detected by immunoblotting (Fig. 7). In control epithelia, Stat1 was located in both cytosolic and nuclear fractions. Following IFN treatment, the nuclear fraction contained the majority of Stat1. In KGF-treated cells in the absence of IFN, Stat1 was located in both cytosolic and nuclear fractions, suggesting that the KGF did not change the subcellular distribution of Stat1. However, KGF prevented IFN-induced trafficking of Stat1 from the cytosol to the nucleus. By inhibiting nuclear trafficking of Stat1, KGF may block the subsequent transcription of IFN-induced genes.

**Fig. 4.** Cluster analysis of genes involved in ion and fluid transport. Genes from the Affymetrix HuFL GeneChip were identified as having roles in ion or fluid transport. Expression patterns of these genes following KGF treatment were analyzed as described in Methods and legend of Fig. 2. CFTR, cystic fibrosis transmembrane conductance regulator; AQP, aquaporin; ENaC, epithelial Na+ channel.

**Fig. 5.** Effect of KGF on Isc in cystic fibrosis (CF) epithelia. Epithelia were studied 24 h after the addition of KGF. Squares indicate control epithelia, and circles indicate KGF-treated epithelia (n = 3 epithelia from each of two different cultures for each condition). KGF decreased the amount of amiloride-sensitive current (26.7 vs. 11.8 μA/cm²; P < 0.001), but did not alter the changes in current that followed other interventions.

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Promotion of epithelial proliferation and repair following injury has been the most investigated role of KGF (41). However, airway injury is often accompanied by inflammation. Thus it is interesting that expression profiling uncovered a decrease in IFN-induced transcripts following KGF treatment. IFN is produced during viral infections and often accompanies inflammation in an injured lung (13). Our data suggest that KGF may reduce the inflammatory effects of IFN. The suggestion is consistent with earlier reports. For example, KGF decreased the levels of inflammatory cytokines in the lungs of mice subjected to bone marrow transplantation (28). In addition, KGF protected animals from inflammatory cell migration into the airway following Pseudomonas infection (38).

Our finding that KGF prevented IFN-induced Stat1 trafficking to the nucleus provides an explanation for how KGF reduced expression of IFN-induced genes. However, our data do not reveal the mechanism by which KGF prevented nuclear trafficking of Stat1. It is possible that KGF altered the phosphorylation state of signaling proteins or changed the expression of yet-unidentified chaperones that mediate trafficking of cytosolic proteins to the nucleus. Identifying the mechanism might provide a potential therapeutic target for modifying the inflammatory response. A previous study using alveolar epithelia showed that surfactant protein A expression increased following KGF treatment (42), providing another potential mechanism for protecting the airway from inflammation. In our microarray studies, surfactant protein A transcript levels

Fig. 6. Cluster analysis of interferon (IFN)-related genes. Genes related to IFN signaling were identified from the Affymetrix HuFL GeneChip and plotted as described in METHODS and legend of Fig. 2. Staf, stimulated trans-acting factor; PA, proteosome activator; ISG, interferon-stimulated gene; IFP, interferon-induced leucine zipper protein; Mx, myxovirus resistance.

Fig. 7. KGF prevents the nuclear localization of Stat1 following IFN stimulation. Control (top) and KGF-treated (bottom) airway epithelia were exposed to 100 U/ml IFN-α and 100 U/ml IFN-γ as indicated. After 30 min of exposure, the cells were lysed, solubilized, and fractionated by centrifugation. Equal amounts of protein were added to each lane. In epithelia not treated with KGF, Stat1 was detected in both the cytosolic and nuclear fractions prior to IFN exposure. Following exposure to IFN, Stat1 was localized predominantly in the nuclear fraction. Bottom shows IFN treatment of KGF-treated epithelia did not change the subcellular distribution of Stat1.
in human airway epithelia did not change with KGF treatment.

Based on the results, we speculate that KGF may have an important role not only in stimulating airway epithelial cell proliferation following injury, but also in reducing inflammation that could further damage the lung. Thus attempts to use KGF therapeutically (12) might benefit from an attempt to monitor anti-inflammatory effects of KGF. The involvement of KGF in the IFN signaling pathway was an unexpected finding. Understanding the full spectrum of functions for KGF and related molecules may also suggest novel applications in understanding lung biology and developing new therapies.

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REFERENCES


