invited review

Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair

LORRAINE B. WARE1 AND MICHAEL A. MATTHAY2
1Division of Pulmonary and Critical Care, Department of Medicine, University of California, Los Angeles 90024; and 2Cardiovascular Research Institute, University of California, San Francisco, California 94143

Ware, Lorraine B., and Michael A. Matthay. Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair. Am J Physiol Lung Cell Mol Physiol 282: L924–L940, 2002; 10.1152/ajplung.00439.2001.—A growing body of evidence indicates that the epithelial-specific growth factors keratinocyte growth factor (KGF), fibroblast growth factor (FGF)-10, and hepatocyte growth factor (HGF) play important roles in lung development, lung inflammation, and repair. The therapeutic potential of these growth factors in lung disease has yet to be fully explored. KGF has been best studied and has impressive protective effects against a wide variety of injurious stimuli when given as a pretreatment in animal models. Whether this protective effect could translate to a treatment effect in humans with acute lung injury needs to be investigated. FGF-10 and HGF may also have therapeutic potential, but more extensive studies in animal models are needed. Because HGF lacks true epithelial specificity, it may have less potential than KGF and FGF-10 as a targeted therapy to facilitate lung epithelial repair. Regardless of their therapeutic potential, studies of the unique roles played by these growth factors in the pathogenesis and the resolution of acute lung injury and other lung diseases will continue to enhance our understanding of the complex pathophysiology of inflammation and repair in the lung.

acute lung injury; acute respiratory distress syndrome; epithelial growth factor

SINCE THE DISCOVERY AND CHARACTERIZATION of the epithelial-specific growth factors keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF), their roles in lung development, lung inflammation, and repair have been widely investigated. Over the past 10 yr, it has become increasingly clear that KGF and HGF play important roles in both the normal and the injured lung and ultimately may have therapeutic potential in lung disease. This review presents a brief history of the discovery and physical properties of KGF and HGF and then focuses on current knowledge of the biological effects of KGF and HGF in lung development and in the injured lung. Fibroblast growth factor-10 (FGF-10), a recently discovered epithelial growth factor with structural and functional similarities to KGF, will also be discussed.

KGF

Background and Basic Properties

Interest in identifying epithelial-specific growth factors that might be oncogenic led to the isolation of KGF from a human embryonic lung fibroblast line by Rubin et al. (104) in 1989. The factor was termed keratinocyte growth factor because of its potent mitogenic activity on mouse epidermal keratinocytes. Subsequent studies showed that KGF is a member of the FGF family (and is also designated FGF-7) and, like other members of the family, has heparin-binding capability (35, 150). Unlike other members of the FGF family, KGF has epithelial specificity; KGF is expressed predominantly by mesenchymal cells, and its receptor (KGF receptor;
KGFR) is expressed only in epithelial cells. This epithelial specificity suggests that KGF may play an important role in mesothelial-epithelial interactions (27).

Attempts to find new FGFs with sequence homology to KGF and other FGFs led to the discovery of FGF-10 in 1996 (157). Initial studies indicated that its sequence had significant homology to KGF and that it was expressed preferentially in the lung of adult rats and rat embryos (157). Like KGF, human FGF-10 is mitogenic for keratinocytes but not fibroblasts (32) and is highly induced in the skin after wounding (125). This similarity to KGF has led some researchers to label it KGF-2. However, in this review it will be referred to as FGF-10.

A comparison of the basic properties and receptor specificity of KGF and FGF-10 is shown in Table 1. Unlike other members of the FGF family that bind to a variety of FGF receptors, KGF binds only to a splice variant of FGF receptor (FGFR) termed FGFR2-IIIb or KGFR (46). Like KGF, FGF-10 binds with high affinity to FGFR2-IIIb but has also been shown to have a weaker affinity for FGFR1-IIIb (8, 53, 55). These receptors are expressed only in epithelial tissues, thus conferring the unique paracrine epithelial specificity of these growth factors. KGF also interacts with low-affinity cell surface heparan sulfate proteoglycan receptors (16). This interaction has a potentiating effect on the interaction of KGF with KGFR (50). Heparan sulfate proteoglycan may also bind to the KGFR, further modulating the KGF-KGFR interaction (50). The interaction of FGF-10 with cell surface heparan sulfate proteoglycan has not been as well studied but is likely similar. FGF-10 does have fourfold higher affinity for pericellular matrix heparan sulfate than KGF (53).

### Role of KGF in the Developing Lung

A role for KGF in lung development was first suggested when it was reported that FGFR2 is expressed in the epithelial cells of the developing lung (97). Targeting a dominant negative FGFR2 to the lung led to the total absence of lung development (96). KGF is expressed in mesenchymal cells of the developing lung and other organs (63). Overexpression of KGF in the mouse lung epithelium either constitutively (116) or conditionally (130) caused embryonic pulmonary malformation with histological similarities to pulmonary cystadenoma (Fig. 1). Embryonic lungs had dilated saccules lined with columnar epithelial cells and no normal alveolar architecture, and the embryos died before reaching term. Studies in explanted rat lungs have provided further evidence for the importance of KGF in lung morphogenesis. Both the addition of exogenous KGF (114) and blocking KGF or KGFR expression using antisense oligonucleotides (100) disrupt normal branching morphogenesis in fetal rat lung explants. The effects of KGF in lung development depend on the stage of gestation. When KGF was expressed in the mouse liver late in gestation using an apolipoprotein E promoter, the predominant effect in the lung was type II cell and bronchiolar cell hyperplasia rather than pulmonary malformation (83). Interestingly, although these studies suggest a role for KGF in normal lung morphogenesis, KGF null mice had histologically normal lung development and survival (41). The lack of an effect of the KGF null mutation can now be partially explained by the discovery that FGF-10 also binds to KGFR and is an important mediator of branching morphogenesis in the developing lung. FGF-10 is highly expressed at the sites where distal buds will appear in the embryonic mouse lung (9).

<table>
<thead>
<tr>
<th>Property</th>
<th>Keratinocyte Growth Factor</th>
<th>Fibroblast Growth Factor-10</th>
<th>Hepatocyte Growth Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass</td>
<td>28 kDa</td>
<td>20 kDa</td>
<td>69-kDa α-chain, 34-kDa β-chain</td>
</tr>
<tr>
<td>Homology</td>
<td>Fibroblast growth factor family</td>
<td>Fibroblast growth factor family</td>
<td>Related to plasminogen, has 4 kringle domains and an inactive serine protease site</td>
</tr>
<tr>
<td>Activation required?</td>
<td>Single chain polypeptide is bioactive</td>
<td>Single chain polypeptide is bioactive</td>
<td>Single chain is cleaved proteolytically to active c-met protooncogene product</td>
</tr>
<tr>
<td>High-affinity receptor</td>
<td>FGFR2-IIIb</td>
<td>FGFR2-IIIb</td>
<td>FGFR1-IIIb</td>
</tr>
<tr>
<td>Receptor type</td>
<td>Membrane-spanning tyrosine kinase</td>
<td>Membrane-spanning tyrosine kinase</td>
<td>Membrane-spanning tyrosine kinase</td>
</tr>
<tr>
<td>Low-affinity receptors</td>
<td>Heparan sulfate proteoglycans</td>
<td>Heparan sulfate proteoglycans</td>
<td>Heparan sulfate proteoglycans</td>
</tr>
<tr>
<td>Cells that produce growth factor</td>
<td>Mesenchymal cells including fibroblasts and vascular smooth muscle cells</td>
<td>Mesenchymal cells</td>
<td>Mesenchymal cells, bronchial epithelial cells, alveolar macrophages</td>
</tr>
<tr>
<td>Target cells that express receptor</td>
<td>Epithelial cells of all types; effects on endothelial cells suggest that an unidentified receptor may be present</td>
<td>Epithelial cells of all types</td>
<td>Primarily epithelial cells of all types but also endothelial cells, fibroblasts, microglial cells, neurons, hematopoietic cells</td>
</tr>
</tbody>
</table>

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FGFR2-IIIb, fibroblast growth factor receptor 2-IIIb.

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components including disaturated phosphatidylcholine and surfactant proteins A, B, and C (26). Similar findings have been reported in a mesenchyme-free lung epithelial culture system where KGF administration promoted distal epithelial differentiation and surfactant protein expression (21, 28). In vivo, intratracheal, intravascular, or intramuscular KGF administration to preterm rabbits significantly increased lung-tissue saturated phosphatidylcholine (47).

Glucocorticoid effects in the fetal lung may also be mediated by KGF. Administration of dexamethasone, known to enhance fetal type II cell maturation and surfactant synthesis, was accompanied by a 50% increase in KGF mRNA in fetal lung fibroblasts (25). In fetal lung explants cultured with dexamethasone, an increase in KGF and KGFR expression was measured along with increases in surfactant protein expression and mature type II cell phenotype (88).

Both KGF and FGF-10 also play an important role in fetal lung fluid secretion, a process that is closely linked to lung morphogenesis. The fetal airway and alveoli actively secrete fluid, and normal lung development is dependent on this process (12). Experimental studies indicate that active chloride secretion is the driving force for fetal lung fluid secretion (49, 70, 84) and that the fetal mesenchyme can produce soluble factors that alter fetal lung distal epithelial ion transport (98). In fetal mouse lung explants, administration of KGF led to increased fluid secretion that was independent of cystic fibrosis transmembrane conductance regulator (CFTR) and could be inhibited by ouabain and bumetanide (165). Similar findings have been reported in the human fetal lung for both KGF and FGF-10 (38). Thus both KGF and FGF-10 appear to enhance CFTR-independent fluid accumulation in the fetal lung. A candidate chloride channel for this effect is CLC-2, a fetal lung epithelial chloride channel that exhibits increased expression on the apical surface of the respiratory epithelium after KGF administration (11). KGF also inhibited expression of the chloride channel (165).

Effects of KGF in the Injured Lung

Endogenous KGF. The role of endogenous KGF in acute lung injury has not been well studied. However, it seems likely, on the basis of the key role that endogenous KGF has been shown to play in wound healing in the skin (60, 152), that endogenous KGF plays an important role in epithelial repair in the lung as well. In neonatal rabbits exposed to hyperoxia, KGF mRNA expression was increased 12-fold in whole lung homogenates at 6 days compared with controls (22). This rise in KGF mRNA was followed, at 8–12 days, by an increase in type II cell proliferation, suggesting that increased expression of KGF led to alveolar epithelial type II cell hyperplasia in response to hyperoxic injury. In a rat model of increased permeability pulmonary

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Fig. 1. Effect of targeted overexpression of human (h) keratinocyte growth factor (KGF) in the murine lung using a surfactant protein C (SP-C) promoter. Comparison of pulmonary development pattern and pulmonary epithelial proliferation and morphology in a normal mouse at gestational day 16.5 (E16.5; A, C, and F) compared with a littermate SP-C-hKGF transgenic embryo (B, D, and F). A: photomicrograph of a sagittal section showing lungs of a normal E16.5 mouse. B: photomicrograph of a sagittal section showing lungs of an E16.5 SP-C-hKGF transgenic mouse lung. C: staining of lungs from A for Ki-67, an endogenous marker of cell proliferation. D: Ki-67 staining of lungs from B. Arrows in C and D point to proliferating cells staining positive for Ki-67 expression. E: higher powered photomicrograph of a distal epithelial airway from A. Note the cuboidal shape characteristic of the distal airway epithelium in normal embryonic mouse lung. F: higher powered micrograph of the epithelial cells lining the large dilated saccules in B. Note the more columnar appearance of the epithelial cells, characteristic of more primordial or immature bronchial epithelium. Sections were stained with hematoxylin and eosin. Bar = 500 μm for A and B; 100 μm for C and D; and 25 μm for E and F. [From Simonet et al. (116).]

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and acts as chemoattractant for epithelial lung buds in concert with KGF (95). FGF-10 null mice have striking abnormalities, including total absence of lung development below the trachea, no limb bud initiation, and other organ abnormalities including a lack of thyroid, pituitary, or salivary glands and malformation of the teeth, kidneys, hair follicle, and gut (73, 86). Thus KGF and FGF-10 have complementary and overlapping roles in the regulation of branching morphogenesis in the lung in concert with several other growth factors and signaling molecules (51, 146).

In addition to its role in lung morphogenesis, KGF has important effects on epithelial differentiation in the developing lung. In isolated rat fetal lung epithelial cells, type II cell maturation and surfactant synthesis appear to be under the control of mesothelial-epithelial interactions. Chelly et al. (25) recently reported that at least one-half of the stimulation of surfactant synthesis by fibroblast-conditioned media in isolated rat fetal lung epithelium could be abrogated by a KGF-neutralizing antibody. Administration of KGF to fetal rat type II cells led to increased synthesis of all surfactant

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edema due to exposure to α-naphthylthiourea (ANTU), pretreatment with a small dose of ANTU leads to resistance to pulmonary edema when a larger dose is administered. Barton et al. (6) showed that a single low dose of ANTU in rats caused an upregulation of KGF gene transcription in the lung, suggesting that KGF-induced hyperplasia might underlie the induced resistance to ANTU. Finally, in rats with acute lung injury due to bleomycin injection, KGF levels in bronchoalveolar lavage (BAL) increased markedly after injury, peaking at 7–14 days, coincident with peak type II cell proliferation (1). Thus in several different injury models, the available evidence indicates that KGF expression is increased after acute lung injury and may be an important endogenous stimulus for alveolar epithelial proliferation and repair. Results from clinical studies are discussed below.

The molecular mechanisms that upregulate the expression of KGF in the setting of lung injury have not been well studied. On the basis of in vitro studies of nonpulmonary fibroblasts, a variety of proinflammatory cytokines have been shown to upregulate KGF mRNA expression and protein translation, although the effects depend on culture conditions and cell lines studied. Proinflammatory cytokines that have a stimulatory effect on KGF expression include interleukin (IL)-1α (24, 128) and -β (17, 24, 128), tumor necrosis factor-α (17, 128), IL-6 (17, 24), transforming growth factor-α (TGF-α) (24), and platelet-derived growth factor-BB (PDGF-BB) (24). Whether lung fibroblasts in vivo also upregulate KGF expression in response to the proinflammatory cytokines present in early acute lung injury should be investigated. Interestingly, in vitro administration of dexamethasone downregulated both constitutive dermal fibroblast expression of KGF (23) and cytokine-stimulated KGF expression (23, 128). Furthermore, glucocorticoid-treated mice had a markedly reduced induction of KGF mRNA after skin injury, despite high levels of serum growth factors and proinflammatory cytokines (18). Although observed in models of skin injury, these findings are of potential interest in the lung because clinical administration of glucocorticoids early in acute lung injury was not beneficial (10).

Exogenous KGF. The protective effect of exogenous KGF in a model of acute lung injury was first reported in 1995 by Panos et al. (90). In that study, rats pretreated intratracheally with 5 mg/kg of recombinant human KGF had far better survival and virtually no histological changes when exposed to 120 h of hyperoxia compared with untreated animals. Intratracheal KGF has since been shown to have a protective effect in a variety of other lung injury models (Table 2). For example, in an acid instillation model (160), pretreatment with intratracheal KGF 72 h before intratracheal acid instillation reduced mortality, histological changes, inflammatory cell influx, procollagen mRNA levels, and hydroxyproline accumulation (Fig. 2). In an ANTU model of increased permeability pulmonary edema (39, 62), pretreatment with KGF reduced alveolar-capillary barrier permeability and pulmonary edema formation. Similar beneficial effects on vascular permeability and pulmonary edema formation have been reported in a rat model of ventilator-induced lung injury (149). Intratracheal KGF has also been shown to ameliorate radiation pneumonitis (163), bleomycin-induced lung injury (122, 162, 163), lung injury from bleomycin and radiation (Fig. 3) (163), and Pseudomonas aeruginosa pneumonia (138), when given before the insult. Recently, intravenous KGF (5 mg/kg) has also been shown to protect against bleomycin- and hyperoxia-induced lung injury in mice (40), even though it stimulated less alveolar epithelial hyperplasia than intratracheal KGF. Finally, subcutaneous administration of KGF in mice ameliorates graft-vs.-host disease (94) and idiopathic pneumonia syndrome (93) in allogeneic bone marrow transplant models.

Several important observations can be made from a comparison of these studies of KGF in lung injury models. First, KGF was protective for a wide variety of mechanisms of lung injury, including direct epithelial injury (e.g., acid aspiration), direct endothelial injury (e.g., ANTU), and T cell-mediated injury (graft-vs.-host disease). Second, the beneficial effects of KGF were apparent on multiple levels from cellular to whole animal. These beneficial effects included reduced or absent histological changes, decreased fibrosis and deposition of collagen precursors, reduced physiological indices of lung injury including vascular permeability and formation of pulmonary edema, and improved survival. Third, in all studies, pretreatment with KGF was necessary for the protective effect. Simultaneous or posttreatment was not efficacious. These observations suggest that the mechanisms by which KGF exerts its protective effects on lung injury are probably multiple, not immediate, and affect multiple cell types within the lung. Putative mechanisms are summarized in Table 3 and will be discussed below.

Mechanisms of protection in acute lung injury. KGF has a wide variety of effects on lung epithelial cells that may mediate its protective effect in acute lung injury. One of the earliest observations was that both in vivo and in vitro administration of KGF cause alveolar epithelial type II cell proliferation (Fig. 4) (34, 92, 136). In vivo, intratracheal administration in rats stimulates reproducible type II cell hyperplasia that peaks at 2–3 days. Proliferation of type II cells is accompanied by migration to cover the alveolar epithelial barrier with type II cells, a process that histologically resembles reactive type II hyperplasia seen in human lungs after an injurious stimulus (3). Bronchial epithelial hyperplasia also occurs in response to KGF, both in vitro (72) and in vivo (72, 136). A similar response to intratracheal KGF has been observed in mice (40, 57). By 7 days after a single intratracheal administration of KGF, the lung parenchyma returns to normal, a process that is mediated by both apoptosis and differentiation to type I cells (33, 34). In this model, type II cell proliferation is accompanied by increased expression of surfactant proteins A, B, and D. Increases in surfactant protein secretion could have several beneficial effects in lung injury, including prevention of alveolar
### Table 2. Summary of studies of keratinocyte growth factor in animal models of acute lung injury

<table>
<thead>
<tr>
<th>First Author (Reference)</th>
<th>Year</th>
<th>Species</th>
<th>Lung Injury Model</th>
<th>KGF, mg/kg</th>
<th>Route</th>
<th>Time</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panos (90)</td>
<td>1995</td>
<td>Rat</td>
<td>Hyperoxia 100% O_2 for 120 h</td>
<td>0.1, 1, or 5</td>
<td>IT</td>
<td>48 h pre</td>
<td>Decreased parenchymal hemorrhage, edema, and mortality in 1- and 5- but not 0.1-mg/kg groups</td>
</tr>
<tr>
<td>Yano (160)</td>
<td>1996</td>
<td>Rat</td>
<td>Acid instillation 0.5 ml 0.1 N HCl into L mainstem bronchus</td>
<td>5</td>
<td>IB to L lung</td>
<td>48 or 72 h pre</td>
<td>48 and 72 h pretreatment improved mortality, but only 72-h group had fewer morphological changes and lower procollagen and hydroxyproline levels, posttreatment was ineffective</td>
</tr>
<tr>
<td>Yi (163)</td>
<td>1996</td>
<td>Rat</td>
<td>Radiation 18 Gy thoracic radiation or bleomycin 2.5 U IT or bleomycin (1.5 U) + radiation (18 Gy)</td>
<td>5</td>
<td>IT</td>
<td>48 or 72 h pre</td>
<td>48 and 72 h pretreatment improved histology but not survival after radiation; 48 and 72 h pretreatment improved survival after bleomycin with no evidence of lung fibrosis; 48 and 72 h pretreatment prolonged survival after combination of bleomycin and radiation</td>
</tr>
<tr>
<td>Mason (62)</td>
<td>1996</td>
<td>Rat</td>
<td>α-naphthylthiourea 50 mg/kg IT</td>
<td>5</td>
<td>IT</td>
<td>48 h pre</td>
<td>KGF group had decreased lung edema, lavage protein, and fewer histopathological changes in isolated perfused lung</td>
</tr>
<tr>
<td>Guery (39)</td>
<td>1997</td>
<td>Rat</td>
<td>α-naphthylthiourea 50 mg/kg IP</td>
<td>5</td>
<td>IT</td>
<td>48 h pre</td>
<td>KGF reduced pulmonary edema and restored alveolar epithelial fluid transport, although it did not protect against increased permeability of the alveolar-capillary barrier</td>
</tr>
<tr>
<td>Guo (40)</td>
<td>1998</td>
<td>Rat</td>
<td>Bleomycin 10 U/kg in rat, 2 U/kg in mice hyperoxia &gt;95% O_2 for 3.5 days</td>
<td>5</td>
<td>IT or IV</td>
<td>48 or 72 h pre</td>
<td>IV or IT KGF improved survival after bleomycin or hyperoxia in mice, 5 mg/kg IV KGF was also effective when given at start of hyperoxia; IT and to a lesser extent IV KGF improved weight loss and respiratory distress after bleomycin in rats</td>
</tr>
<tr>
<td>Panoskaltsis-Mortari (94)</td>
<td>1998</td>
<td>Mice</td>
<td>GVHD allogeneic bone marrow transplant</td>
<td>5</td>
<td>SC</td>
<td>−6, −5 and −4 day pre</td>
<td>KGF improved survival, and GVHD-induced tissue damage in the lung and other organs</td>
</tr>
<tr>
<td>Sugahara (122)</td>
<td>1998</td>
<td>Rat</td>
<td>Bleomycin 5 mg/kg</td>
<td>150 μg/kg</td>
<td>IT</td>
<td>48 h pre and 24 h post</td>
<td>Two doses of KGF prevented weight loss and reduction in total lung capacity, attenuated protein accumulation in the lung, and reduced expression of collagen I and III compared with controls</td>
</tr>
<tr>
<td>Yi (162)</td>
<td>1998</td>
<td>Rat</td>
<td>Bleomycin 1.5 U IT</td>
<td>5</td>
<td>IT</td>
<td>72 h pre</td>
<td>At 3 days after bleomycin, pretreatment with KGF preserved the number of type II cells and Clara cells and reduced BAL protein, TGF-β, and PDGF-BB compared with controls</td>
</tr>
<tr>
<td>Panoskaltsis-Mortari (93)</td>
<td>2000</td>
<td>Mice</td>
<td>Idiopathic pneumonia syndrome after allogeneic bone marrow transplant</td>
<td>5</td>
<td>SC</td>
<td>−6, 5 and 4 days pre</td>
<td>KGF-treated mice had alveolar type II cell hyperplasia at day 3 posttransplant, failed to upregulate cytolytic molecules, and had induction of anti-inflammatory Th2 cytokines compared with controls</td>
</tr>
<tr>
<td>Welsh (149)</td>
<td>2000</td>
<td>Rat</td>
<td>Ventilator-induced 17 ml/kg tidal volume and PEEP = 0 cm H_2O</td>
<td>1</td>
<td>IV</td>
<td>Daily for 72 h pre</td>
<td>KGF group had less pulmonary edema, improved dynamic compliance, and reduced alveolar protein accumulation</td>
</tr>
<tr>
<td>Viget (138)</td>
<td>2000</td>
<td>Rat</td>
<td>Bacterial pneumonia Pseudomonas aeruginosa</td>
<td>5</td>
<td>IT</td>
<td>48 h pre</td>
<td>KGF restored normal alveolar epithelial fluid transport, decreased bacterial pulmonary translocation, and increased BAL neutrophils compared with controls</td>
</tr>
</tbody>
</table>

**Abbreviations:** KGF, keratinocyte growth factor; IT, intratracheal; L, left; IB, intrabronchial; IP, intraperitoneal; IV, intravenous; SC, subcutaneous; GVHD, graft-vs.-host disease; BAL, bronchoalveolar lavage; PEEP, positive end-expiratory pressure, TGF-β, transforming growth factor-β; PDGF-BB, platelet-derived growth factor-BB.
collapse by reduction of alveolar surface tension and augmentation of host defense. However, whereas in vitro KGF administration enhances surfactant protein expression on a per cell basis (112, 124), in vivo KGF administration enhances surfactant protein expression only on a whole lung basis. Individual type II cell levels of surfactant protein expression are decreased (161). The differential effect of KGF in vitro compared with in vivo probably has multiple explanations, including relative differences in dose and duration of exposure to KGF as well as complex environmental influences in vivo that are not present in a simple in vitro system.

When KGF expression is increased via adenovirus-mediated gene transfer either in vitro in rat type II cells or in vivo, similar findings of type II cell hyperplasia and increased surfactant proteins A and D production have been reported (78).

One of the histological hallmarks of acute lung injury is sloughing and necrosis of the alveolar epithelium (2, 3). In addition to type II cell proliferation, regeneration of a normal alveolar epithelium requires migration of type II cells along the denuded basement membrane to reconstitute an intact epithelium (143). KGF enhances the spreading and motility of alveolar epithelial type II cells, suggesting that improved alveolar repair may underlie some of the protective effects of KGF in lung injury. The beneficial effects of KGF on wound closure were first observed in the skin (120, 151, 152), bladder (7), gastric epithelium (126), and cornea (119). In the lung, KGF enhances wound closure during cyclic mechanical strain in bronchial epithelial monolayers through enhanced cell spreading and motility (144). KGF also enhances the alveolar epithelial repair activity of rat alveolar epithelial type II cells when administered in vivo (141) or in vitro (M.-P. d’Ortho, unpublished observations). Altered migration and wound repair in KGF-treated epithelial cells may, in part, be a function of altered interaction with the extracellular matrix through increased expression of matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (UPA). These effects have not yet been studied in lung epithelial cells. In cultured human keratinocytes, KGF increased cell migration, UPA activity (108, 132), and MMP-10 (stromelysin-2) activity (58). In porcine periodontal ligament epithelial cells, KGF increased both MMP-13 (collagenase-3) activity (135), MMP-9 (gelatinase) activity, and UPA activity (101).

In addition to enhancing epithelial repair after a mechanical injury, KGF renders epithelial cells more resistant to mechanical injury. KGF-treated alveolar epithelial cells are inherently more resistant to injury from mechanical deformation (Fig. 5) (89), a factor that may contribute to KGF’s protective effects in ventilator-induced lung injury (149). KGF also renders airway epithelial cells more resistant to both hydrogen perox-
ide- (145) and radiation-induced increases in cell permeability (109). Interestingly, in both of these in vitro studies, the protective effect was not limited strictly to pretreatment. In the radiation model, KGF was partially protective when given immediately after radiation (109). In the hydrogen peroxide model, as little as 1 h of pretreatment with KGF was partially protective, suggesting that at least some of the KGF effects were posttranslational (145). In both these studies, KGF’s protective effects were associated with stabilization of the F-actin cytoskeleton and could be inhibited by blocking protein kinase C. KGF also renders alveolar epithelial cells more resistant to cell necrosis induced by in vitro mechanical deformation (89). In that study, KGF treatment accelerated the in vitro transition from type II phenotype to type I phenotype, a transition that may have conferred resistance to mechanical deformation; changes in the actin cytoskeleton and the secretion of extracellular matrix may have also played a role in that model.

KGF also has effects on alveolar epithelial fluid transport in the adult lung. Unlike the fetal lung, which is a net secretor of fluid, the adult lung actively removes fluid from the alveolar space to maintain the gas exchange interface. Alveolar epithelial fluid transport is driven primarily by the active transport of sodium across the alveolar epithelial barrier by alveolar epithelial type II cells (68). The ability to clear fluid from the alveolar space is critically important to the resolution of pulmonary edema and to the restoration of adequate gas exchange in the setting of acute lung injury (142). Furthermore, in both animal models of acute lung injury and clinical acute lung injury, alveolar fluid clearance is impaired (69, 76, 77, 142). KGF has been shown to increase alveolar epithelial fluid transport in both in vitro and in vivo studies in both the uninjured and the injured lung. In primary isolates of rat alveolar epithelial cells, addition of KGF enhanced active ion transport across monolayers primarily due to increased Na-K-ATPase α₁-subunit expression (13). In the normal rat lung, intratracheal pretreatment with KGF increased alveolar epithelial fluid transport both in vivo (140) and in the isolated perfused lung (39). The primary mechanism was by type II cell hyperplasia since expression of the ENaC was diminished on a per cell basis (140). In rats with lung injury due to P. aeruginosa pneumonia, KGF pretreatment prevented the reduction in alveolar epithelial fluid transport observed in the untreated animals (138). Similar findings have been reported after ANTU injury in an isolated perfused lung model (39).

Table 3. Possible mechanisms to explain the protective effects of KGF and HGF in acute lung injury

<table>
<thead>
<tr>
<th></th>
<th>KGF Reference No.</th>
<th>HGF Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects on alveolar type II and airway epithelial cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased proliferation</td>
<td>(34, 72, 92, 136)</td>
<td></td>
</tr>
<tr>
<td>Increased surfactant protein and phospholipid production</td>
<td>(112, 124, 161)</td>
<td></td>
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<tr>
<td>Enhanced spreading and motility</td>
<td>(141)</td>
<td></td>
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<tr>
<td>Altered release of matrix metalloproteinases and urokinase-type plasminogen activator</td>
<td>(58, 101, 108, 132, 135)</td>
<td>(30)</td>
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<tr>
<td>Resistance to mechanical injury</td>
<td>(89)</td>
<td></td>
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<tr>
<td>Resistance to oxidant-induced injury</td>
<td>(109, 144)</td>
<td></td>
</tr>
<tr>
<td>Enhanced alveolar epithelial fluid transport</td>
<td>(13, 39, 138, 140)</td>
<td>(133, 159)</td>
</tr>
<tr>
<td>Enhanced DNA repair</td>
<td>(20, 127, 153)</td>
<td></td>
</tr>
<tr>
<td>Decreased apoptosis</td>
<td>(20, 54)</td>
<td></td>
</tr>
<tr>
<td>Release of cytokines and growth factors with autocrine and paracrine effects</td>
<td>(29, 42, 141, 162)</td>
<td>(133, 159)</td>
</tr>
<tr>
<td><strong>Other effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhanced endothelial cell barrier function, motility, and resistance to injury</td>
<td>(6, 36)</td>
<td>(117)</td>
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HGF, hepatocyte growth factor.
The effects of KGF on alveolar epithelial fluid transport can be additive with other measures to stimulate alveolar fluid clearance. In one study, KGF treatment combined with the cAMP agonist terbutaline more than doubled the rate of alveolar fluid clearance (140) (Fig. 6).

Several groups have investigated the effects of KGF on DNA repair after oxidant injury. In A549 alveolar epithelial cells exposed to radiation, addition of KGF to the media ameliorated the formation of DNA strand breaks. This protective effect of KGF was blocked by the addition of inhibitors of DNA polymerases α, δ, and ε, indicating that the effect was due to enhanced DNA repair (127). Similar findings were reported when A549 or primary isolates of rat alveolar epithelial cells were exposed to H₂O₂. Again, the protective effect of KGF against DNA strand breaks was blocked by the addition of inhibitors of DNA polymerases (α, β, δ, and ε) in this study (153). When rats were exposed to hyperoxia and allowed to recover before isolation of alveolar epi-

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**Fig. 4.** Schematic diagram shows the progression of type II pneumocyte hyperplasia in the lung after a single intratracheal injection of KGF (5 mg/kg). [From Ulich et al. (136).]

**Fig. 5.** Enhanced viability after application of mechanical strain in KGF-treated rat alveolar epithelial cells. Rats were given KGF (5 mg/kg intratracheal) 48 h before isolation of alveolar epithelial type II cells. Cytoplasm of viable cells was stained using calcein-AM (indicated in green), and nuclei of nonviable cells were stained using ethidium homodimer-1 (indicated in red). A: saline vehicle-treated unstretched cells. B: saline vehicle-treated cells after 1 h of cyclic stretch (25% change in surface area). C: KGF-treated unstretched cells. D: KGF-treated cells after same 1-h stretch protocol. [From Oswari et al. (89).]

**Fig. 6.** Effect of terbutaline on alveolar liquid clearance over 1 h in rats 72 h after KGF treatment (5 mg/kg intratracheal). Data are also shown for alveolar liquid clearance over 1 h in control rats and in control rats treated with 10⁻⁴ M terbutaline. Data are means ± SD. *P < 0.05 compared with control group, †P < 0.05 compared with 72 h after KGF treatment and P < 0.05 compared with terbutaline control. Top: %stimulation (means ± SD) by terbutaline with and without KGF. [From Wang et al. (140).]
thelial cells, DNA strand break formation was observed in culture that could be blocked by adding KGF to the culture medium (20). This protective effect was associated with the appearance of proliferating cell nuclear antigen, suggesting that the KGF may facilitate transition to a point in the cell cycle where DNA strand breaks can be repaired. KGF may also prevent epithelial cells from responding to proapoptotic stimuli. In mice, KGF protected against hepatocyte apoptosis induced by injection of lipopolysaccharide and d-galactosamine (111). Although there have been only a few studies in the lung, KGF appears to inhibit hyperoxia-induced apoptosis of alveolar epithelial cells (20, 54), probably through induction of an antiapoptotic pathway, the Akt signaling axis (54).

Although KGF has a multitude of direct effects on epithelial cell proliferation, motility, fluid transport, and repair, some of the beneficial effects of KGF may be mediated by the release of downstream mediators that have both autocrine and paracrine effects. For example, in a rat model of T cell-mediated idiopathic pneumonia after bone marrow transplantation, KGF administration before bone marrow transplant suppressed T cell-dependent alveolar macrophage activation and production of inflammatory mediators (42). This finding suggests that KGF stimulated the release of an epithelial cell-derived mediator capable of downregulating macrophage function. The protective effect of KGF in this model was blocked if a nitrating species was introduced by adding cyclophosphamide to the conditioning regimen. The authors hypothesize that the generation of peroxynitrite disabled downstream signaling from the KGF receptor by disabling tyrosine phosphorylation (42). KGF may also stimulate epithelial cells to produce other growth factors. In cultured murine keratinocytes, KGF stimulated the expression and secretion of TGF-α into the medium (29). Similarly, supernatants from alveolar epithelial cells isolated from KGF-treated rats stimulated alveolar epithelial repair (141), consistent with an autocrine effect of KGF. This effect also appears to be mediated through the epidermal growth factor (EGF) receptor (K. Atabai, unpublished observations), perhaps due to the stimulation of production of soluble factors such as EGF or TGF-α. KGF treatment may also prevent the release of potentially harmful mediators. In a bleomycin-induced lung injury model in rats, pretreatment with KGF prevented the bleomycin-associated increase in profibrotic mediators including TGF-β and PDGF-BB (162).

The majority of studies of the protective effect of KGF in acute lung injury have focused on epithelial cells. However, a few recent reports indicate that KGF may have direct or indirect effects on endothelial cells that contribute to protection from acute lung injury. Gillis et al. (36) reported that subnanomolar concentrations of KGF induced neovascularization in the rat cornea. In this study, KGF induced chemotaxis in capillary but not large vessel endothelial cells in culture. FGF-10 had similar effects. KGF also helped to maintain the barrier function of capillary endothelial cell monolayers, protecting against hydrogen peroxide- and vascular endothelial growth factor-induced increases in permeability. However, KGFR could not be localized to endothelial cells. The authors hypothesize that KGF may be acting through an as yet undiscovered high affinity receptor on endothelial cells since KGF administration led to rapid rise in mitogen-activated protein kinase activity in capillary endothelial cells (36). A protective effect of KGF on the endothelium was also suggested in an in vivo hyperoxia study. In this study, KGF administration prevented damage by hyperoxia to both the alveolar epithelium and capillary endothelium as measured by electron microscopy (5). The mechanism of the protective effect for the endothelium was not defined, although whole lung levels of the cell death-associated proteins p53, Bax, and Bcl-x all declined, as did levels of plasminogen activator inhibitor-1. In an isolated perfused rat lung model, intravenous KGF attenuated hydrostatic pulmonary edema, a finding that was associated with decreased alveolar-capillary barrier permeability and may have been due to effects on endothelial permeability (148).

Clinical Studies of KGF in the Lung

There have been very few clinical studies evaluating the role of endogenous KGF in human acute lung injury. Verghese et al. (137) measured levels of KGF in undiluted pulmonary edema fluid sampled from patients with early acute lung injury. Although KGF was detected (0.3–2.1 ng/ml) and was bioactive, there was no difference in levels in patients with acute lung injury compared with control patients with hydrostatic pulmonary edema. However, because KGF is a heparin-binding protein, measurements in the soluble phase may not be the optimal way to detect changes in KGF expression after lung injury in humans. Stern et al. (121) collected BAL fluid from patients with acute respiratory distress syndrome (ARDS) later in their course than in the Verghese study. KGF was detected in BAL fluid in 13 of 17 patients with ARDS vs. only 1 of 8 patients with hydrostatic pulmonary edema. Mechanically ventilated patients without ARDS or hydrostatic edema did not have detectable levels of KGF in BAL. Detectable levels of KGF were associated with measurable levels of type III procollagen peptide and death, but only when both ARDS and non-ARDS patients were considered together.

The use of exogenous KGF as a treatment for human acute lung injury has not been studied. Because KGF has only been effective as a pretreatment in animal models, there has been little enthusiasm for clinical trials in acute lung injury. In humans, the development of acute lung injury is rarely predictable. Thus a preventive therapy lacks appeal. However, it should be noted that the available animal models of acute lung injury do not adequately reproduce the clinical situation. In humans with acute lung injury, ventilatory and hemodynamic support along with treatment for the underlying inciting clinical disorder may provide a prolonged interval for KGF to exert its therapeutic effects, a situation that cannot be reproduced in animal
models. Furthermore, there are some patients for whom a preventive therapy might be useful such as patients receiving chemotherapy and/or radiation therapy with potential lung toxicity. Although KGF is not currently being evaluated as a treatment for clinical acute lung injury, other therapeutic uses are being explored. Phase I/II studies are underway to evaluate recombinant human KGF as an agent to prevent oral and gastrointestinal mucositis. In a phase I trial in healthy volunteers, a 3-day administration of systemic KGF was safe, well tolerated, and induced a dose-dependent increase in oral mucosal proliferation (27). Topical KGF is also undergoing study for acceleration of wound healing in the skin. Although FGF-10 has shown promise in animal models of gastrointestinal mucositis and wound healing in the skin, it is still in preclinical evaluation.

HEPATOECTY GROWTH FACTOR

Background

The identification of HGF was the result of a concerted effort to identify the growth factor responsible for hepatic regeneration after hepatectomy (102). Initially isolated from multiple sources (37, 71, 80, 105), it was later recognized that HGF was identical to another growth factor, scatter factor, which had been independently isolated and cloned (147). Like KGF, HGF has heparin-binding capability, but it is not a member of the FGF family. HGF is expressed as a single chain molecule of 728 amino acids that is cleaved proteolytically to an active heterodimer (14). The active heterodimer has four kringle domains and an inactive serine protease site and belongs to a group of fibrinolytic and coagulation-related proteins, which includes plasminogen and other blood proteases (14). The HGF receptor (Table 1) is a membrane-spanning tyrosine kinase that was identified as the c-met protooncogene product in 1991 (15, 81). Unlike the KGFR, c-met expression is not confined to the epithelium, although epithelial expression predominates. In addition to normal epithelial cells of almost every organ, c-met has been detected on fibroblasts, endothelial cells, microglial cells, neurons, and hematopoietic cells. Like KGF, HGF binds to cell surface heparan sulfate proteoglycans (56) that serve as low-affinity receptors and modulate the interaction between HGF and the c-met receptor (103, 107).

Role of HGF in the Developing Lung

HGF and its receptor are expressed in many developing organs. HGF expression is usually confined to the mesenchyme, and HGF receptor expression is usually confined to the epithelium (118). HGF null mice die in utero due to abnormalities of the liver and placenta (110, 134). However, lung development is normal at the time of death in these embryos. Brinkmann et al. (19) tested the effect of HGF on various epithelial cell lines and found that HGF could induce endogenous morphogenetic programs in epithelial cells from a variety of organs including the lung (LX-1 carcinoma cells). In embryonic rat lung organoids grown on three-dimensional collagen matrices, antisense HGF oligonucleotides blocked alveolar and bronchial morphogenesis (48). In rat fetal lung explants, exogenous HGF stimulated branching organogenesis (85). However, when fetal lung epithelial explants were grown in the absence of mesenchyme, HGF alone was insufficient to restore branching morphogenesis, whereas KGF alone or acidic FGF alone was sufficient. HGF had a synergistic effect with KGF or acidic FGF in this mesenchyme-free system (85). Thus while HGF appears to play an important role in branching morphogenesis in the lung, it is not essential, perhaps due to redundancy in the repertoire of mediators of mesenchymal-epithelial interactions.

In humans, amniotic fluid from women up to 31 wk pregnant had a motogenic effect on a fetal feline lung cell line (48). This motogenic activity could be abolished by anti-HGF neutralizing antibodies, suggesting that HGF is present and probably functional in human lung development as well. After 31 wk, human amniotic fluid was no longer motogenic for fetal lung cells.

Effects of HGF in the Injured Lung

Endogenous HGF. HGF is present in the BAL fluid of normal adult rats and is responsible for most of the mitogenic effects of lavage fluid on alveolar epithelial cells (65). In the first published study to examine the effect of acute lung injury on HGF expression in the lung, Yanagita et al. (158) reported that HGF mRNA and HGF activity increased in whole lung at 3–6 h after injury with intratracheal hydrochloric acid. This increase in HGF expression was followed at 24 h by a peak in bronchial epithelial DNA synthesis and at 48 h by a peak in alveolar epithelial DNA synthesis. An increase in whole lung HGF expression has also been reported in a rat model of ischemia-reperfusion. In that model, whole lung HGF mRNA increased by 24 h after ischemia-reperfusion. This was followed by an increase in whole lung HGF protein that peaked at day 3 after ischemia-reperfusion. Administration of an anti-HGF antibody aggravated ischemia-reperfusion lung injury and reduced postinjury DNA synthesis in the lung, suggesting that endogenous HGF plays a role in the reparative response to lung injury. In a recent study, Morimoto et al. (79) attempted to localize the cellular source of HGF in a rat model of P. aeruginosa pneumonia. Whole lung HGF mRNA increased at 3 h after bacterial instillation and again at 24–72 h. Immunohistochemistry suggested that the cellular source of HGF for the early peak was bronchial epithelial cells. This finding is surprising and was not confirmed by in situ hybridization but is in keeping with a report that normal human bronchial epithelial cells can produce HGF in culture as an autocrine motogenic factor (131). The cellular source for
the later peak of HGF production appeared to be alveolar macrophages and, in particular, those that had phagocytosed apoptotic neutrophils (79). Fibroblasts isolated from rats exposed to hyperoxia also have increased HGF expression (139).

The lung may also be a source of HGF after injury to other organs. For example, after partial hepatectomy, unilateral nephrectomy, or induction of hepatitis in rats, HGF mRNA in the intact lung increased at 6 h (159). In the setting of acute pancreatitis in rats, HGF mRNA and protein increased in the lung, liver, and kidney (133). These findings suggest that the lung may contribute to organ repair and regeneration in an endocrine fashion through production of circulating HGF.

The factors that lead to upregulation of HGF expression in the setting of lung injury or other organ injury have not been fully elucidated. The human HGF gene has an IL-6 response element and a potential binding site for nuclear factor IL-6 near the transcription initiation site, suggesting that IL-6 may promote transcription (74). This is potentially important because plasma levels of IL-6 are elevated in patients with acute lung injury (129). IL-1α and IL-1β have both been shown to increase HGF mRNA in cultured human skin fibroblasts (66). In addition to transcriptional regulation, local proteolytic activation of HGF may control its activity. Miyazawa and coworkers (75) showed that an enzymatic activity that proteolytically activated the HGF precursor could be induced in the liver in response to tissue injury.

**Exogenous HGF.** Compared with KGF, there have been relatively few studies of the effect of exogenous HGF in lung injury. In bleomycin-induced lung injury in the mouse, concomitant treatment with a continuous infusion of HGF repressed fibrotic morphological changes at 2 or 4 wk after initiation of bleomycin (Fig. 7) (154). Interestingly, HGF infusion was also effective if it was started 2 wk after the bleomycin was started, suggesting that HGF may be able to reverse some of the fibrotic changes induced by bleomycin. Dohi et al. (30) reported that administration of HGF to A549 alveolar epithelial cells in culture enhanced cell surface plasmin generation and expression of urokinase activity, thus enhancing the fibrinolytic capacity of this cell line.

**Clinical Studies of HGF**

To date, the only clinical studies of HGF in lung disease have focused on measuring HGF in biological fluids such as serum, BAL fluid, or pulmonary edema fluid in patients with various lung diseases. In patients with either idiopathic pulmonary fibrosis or collagen vascular disease-associated pulmonary fibrosis, both serum (59, 156) and BAL fluid (106) levels of HGF were elevated. Patients with bacterial pneumonia also had elevated serum levels of HGF (59), although in one study, levels in nonsurvivors were normal (82). Elevated serum HGF levels have also been measured in patients with clubbing (44), after thoracotomy with unilateral ventilation (155), or after pneumonectomy (123).

In patients with acute lung injury, both BAL fluid levels (121) and undiluted pulmonary edema fluid levels (137) of HGF are elevated, and higher levels are associated with increased mortality (Fig. 8). This association with increased mortality does not imply causality but rather may indicate that high levels of HGF in the lung are associated with more severe lung injury and inflammation and thus a worse outcome. Pulmonary edema fluid levels were sevenfold higher than simultaneous plasma levels, indicating some local production of HGF in the lung (137). Thus...
although there is ample evidence that lung disease can increase HGF levels in biological fluids, the clinical studies to date have not explored the mechanistic role of HGF in human lung disease nor have there been any therapeutic trials.

CONCLUSIONS

The epithelial-specific growth factors KGF, FGF-10, and HGF are important mediators of mesenchymal-epithelial interactions during lung development, lung inflammation, and lung repair. Whether these growth factors will also have therapeutic use in lung disease is not yet clear and requires further study. Regardless of any therapeutic potential, future studies of KGF, FGF-10, and HGF will undoubtedly deepen our understanding of the pathogenesis and resolution of acute lung injury and other lung diseases.

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