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Pulmonary malformation in transgenic mice expressing human keratinocyte growth factor in the lung

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ABSTRACT **Expression of human keratinocyte growth factor (KGF/FGF-7) was directed to epithelial cells of the developing embryonic lung of transgenic mice disrupting normal pulmonary morphogenesis during the pseudoglandular stage of development. By embryonic day 15.5 (E15.5), lungs of transgenic surfactant protein C (SP-C)-KGF mice resembled those of humans with pulmonary cystadenoma. Lungs were cystic, filling the thoracic cavity, and were composed of numerous dilated saccules lined with glycogen-containing columnar epithelial cells. The normal distribution of SP-C proprotein in the distal regions of respiratory tubules was disrupted. Columnar epithelial cells lining the papillary structures stained variably and weakly for this distal respiratory cell marker. Mesenchymal components were preserved in the transgenic mouse lungs, yet the architectural relationship of the epithelium to the mesenchyme was altered. SP-C-KGF transgenic mice failed to survive gestation to term, dying before E17.5. Culturing mouse fetal lung explants in the presence of recombinant human KGF also disrupted branching morphogenesis and resulted in similar cystic malformation of the lung. Thus, it appears that precise temporal and spatial expression of KGF is likely to play a crucial role in the control of branching morphogenesis during fetal lung development.**

The mouse embryonic lung buds from the foregut endoderm on embryonic day 9.5 (E9.5) (1). Subsequent development of the respiratory epithelium (E9.5–E14) involves a period of extensive branching morphogenesis, during which columnar cells of the respiratory epithelium line secondary and tertiary airways and are surrounded by a loosely packed mesenchyme (1–3). During the latter embryonic and early postnatal periods, the pulmonary mesenchyme thins and the terminal lung buds undergo sacculation, forming the functional alveolar gas-exchange unit (the alveolar sac) characteristic of a mature lung. The columnar cells lining the maturing embryonic lung become cuboidal as the distal saccules form (1–4). Factors derived from the pulmonary mesenchyme play important roles in stimulating proliferation and differentiation of the developing pulmonary epithelium (5).

Keratinocyte growth factor (KGF/FGF-7), a member of the fibroblast growth factor (FGF) family, has been implicated as an important stroma-derived mediator of epithelial cell growth, having no mitogenic activity on cultured endothelial cells or fibroblasts (6–10). KGF was originally purified from the conditioned medium of a human embryonic lung fibroblast cell line (6). Subsequently, KGF was identified as a potent growth factor stimulating proliferation of cultured primary alveolar type II cells (11). KGF is produced by adult human lung fibroblasts (11) and is expressed in mesenchyme of the embryonic lung at sites of active branching morphogenesis (12). Intratracheal administration of recombinant human KGF (hKGF) to the lungs of adult rats results in a dose-dependent proliferation of alveolar type II pneumocytes (13). Expression of a dominant-negative form of the FGF receptor type 2 variant IIIb (FGFR2-IIIb) (which binds some members of the FGF family including KGF and acidic FGF) in the primordial pulmonary epithelium of transgenic mice completely blocked airway branching and epithelial differentiation (14), suggesting an important role for members of the FGF family, and perhaps KGF specifically, in branching morphogenesis of the lung. These observations are consistent with the hypothesis that KGF may be an integral part of epithelial–mesenchymal interactions that mediate normal lung growth and development (12) and are critical to restoration of alveolar architecture following lung injury.

To directly and specifically assess the role of KGF overexpression on lung development, we used the human surfactant protein C (SP-C) gene promoter (2, 15–17) to target expression of hKGF to the primordial respiratory epithelium of transgenic mice.

MATERIALS AND METHODS

Transgene Preparation. A hKGF cDNA lacking upstream ATGs but including an altered Kozak sequence, CCACC, immediately upstream of the initiating ATG was ligated into the *Eco*RI site of the human SP-C promoter plasmid SP-C 3.7 t intron poly(A) (2, 17, 18). The orientation of the cDNA was verified by sequencing. Plasmid DNA was prepared by the alkaline lysis method and purified through two rounds of CsCl density gradient centrifugation.

Preparation and Analysis of Transgenic Mice. For microinjection, the SP-C-hKGF construct was digested with *Aat* II/*Not* I, and the 4.8-kb transgene insert was purified on a 0.8% ultrapure DNA agarose gel (BRL) and diluted to 1 μg/ml in 5 mM Tris·HCl, pH 7.4/0.2 mM EDTA. Single-cell embryos from (BD × BD)F_1 bred mice were injected essentially as described (19), except that injection needles were beveled and treated with silicon before use. Embryos were cultured overnight in a CO_2 incubator and 15–20 two-cell embryos were transferred to the oviducts of pseudopregnant CD1 female mice. Embryos were considered to be at 1.5 days of gestational development on the day of implantation. The surrogate mothers were sacrificed between 12.5 and 17.5 days of gestation and embryos were harvested for analysis. Transgenic founder embryos, averaging 21% of the littermates derived from implanted zygotes, were identified by PCR analysis of tail DNA using oligonucleotide primers that amplified a 235-bp fragment of the hKGF cDNA.

Histological Examination and Immunostaining of Embryos. For histology and immunostaining, embryos were fixed overnight in 10% neutral buffered zinc formalin (Anatech, Battle

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Abbreviations: KGF, keratinocyte growth factor; hKGF, human KGF; FGF, fibroblast growth factor; proSP-C, surfactant protein C proprotein; E, embryonic day; RT, room temperature.
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Creek, MI), paraffin embedded, and sectioned 3–5 μm thick. Embryo sections were stained with hematoxylin and eosin for routine histologic examination. Sections selected for immunostaining were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 3% H_2O_2 in methanol for 15 min at room temperature (RT). All sections were then blocked with 2% normal goat serum in phosphate-buffered saline (PBS) with 0.2% Triton X-100 for 2 h at RT before incubating overnight at 4°C with the primary antibody. The anti-SP-C proprotein (proSP-C) rabbit polyclonal antibody was generated to a fusion protein containing glutathione *S*-transferase and amino acids 1–20 from the N terminus of the human proSP-C and affinity purified against the same fusion protein (20). After overnight incubation with the primary antibody, the sections were washed six times, 5 min each, in PBS with 0.2% Triton X-100 and then incubated with biotinylated goat anti-rabbit antibody (diluted 1:200 in blocking solution) for 30 min at RT. Sections were incubated with the avidin–biotin–peroxidase complex from the Vectastain Elite ABC peroxidase rabbit IgG kit (Vector Laboratories) diluted in blocking solution for 30 min at RT. The enzymatic reaction product was enhanced with nickel cobalt to give a black precipitate, and the sections were counterstained with nuclear fast red. For detection of Ki-67, an endogenous marker of cell proliferation (21), sections of embryos were first steam heated for 20 min in 10 mM citrate buffer (pH 6) for antigen retrieval, according to a protocol provided by BioTek Solutions (Santa Barbara, CA), and then immunostained with rabbit polyclonal anti-Ki-67 antiserum (Dako). Ki-67 antigen was detected with a biotinylated anti-rabbit secondary antibody (BioTek Solutions) and avidin–biotin complex tertiary antibody (BioTek Solutions) at the recommended dilutions. Staining was visualized with diaminobenzidine (Sigma), and sections were lightly counterstained with hematoxylin. Cellular glycogen content was assessed by periodic acid/Schiff staining with and without diastase pretreatment.

***In Situ* Hybridization.** In preparation for *in situ* hybridization to detect expression of the hKGF transgene, embryos were immersion fixed in 4% paraformaldehyde for 6–8 h at 4°C and cryoprotected for 12–16 h in 15% sucrose. Embryos were embedded in Tissue-Tek OCT compound (Miles), frozen on dry ice, and stored at −80°C. Saggital sections (9 μm thick) were attached to Super Frost slides (Fisher) and stored at −80°C until prehybridization. Hybridization, washing, and exposure to emulsion were performed as described (22). Antisense and sense RNA probes to hKGF were transcribed with viral RNA polymerases in the presence of UTP[^{35}S] from a hKGF cDNA fragment cloned into the transcription vector pSP72 (Promega). The hKGF antisense transcript corresponded to bases 468–674 of the hKGF cDNA (accession no. M60828).

RESULTS

Embryonic Lethality in Mice Overexpressing KGF in the Developing Lung. The SP-C-hKGF DNA was microinjected into fertilized mouse oocytes derived from BD F_1 matings (19). Among 72 potential adult founder mice screened by PCR, only 1 contained the transgene integrated into the genome, and this mouse failed to express hKGF in any tissue analyzed (data not shown). Subsequent analysis of transgenic founder embryos isolated during the latter 1/3rd of the mouse gestational period revealed an embryonic lethal phenotype that correlated with the presence of the transgene (Table 1). Of 32 embryos taken at E17.5, 5 were found to harbor the SP-C-hKGF transgene in their genome. All 5 of these transgenic embryos were dead *in utero* and exhibited variable tissue autolysis and pale extremities. One embryo had marked pulmonary hemorrhage. In contrast, all nontransgenic embryos from E12.5 to E17.5 as well as 22 of 23 transgenic embryos analyzed at or before E15.5 were viable, appearing normal in size and external morphology upon gross observation. The observed pattern of lethality suggests that transgenic embryos were dying between E15.5 and E17.5.

Table 1. Summary of SP-C-hKGF transgenic mouse embryo viability

Age	No. of embryos analyzed	No. of transgenic embryos	No. of viable transgenic embryos
E12.5	28	2	2
E13.5	19	5	5
E14.5	41	5	5
E15.5	38	11	10
E16.5	25	11	6
E17.5	32	5	0

Embryos were harvested from surrogate mothers at the indicated gestational stage. Transgenic embryos and their nontransgenic littermates were identified by a PCR-based screening method and processed as described. Embryonic development was timed from the day of two-cell embryo implantation, which was taken as E1.5. Litter sizes ranged from 2 to 10, exhibiting normal variation at different gestational ages. All nonviable embryos exhibited severe necrosis, had extremely pale extremities, and had grossly autolyzed internal organs.

Abnormal Lung Development in SP-C-hKGF Transgenic Mouse Embryos. Histological examination of saggital cross sections taken from viable E12.5–E16.5 mouse embryos revealed distinct morphological changes in the developing lungs

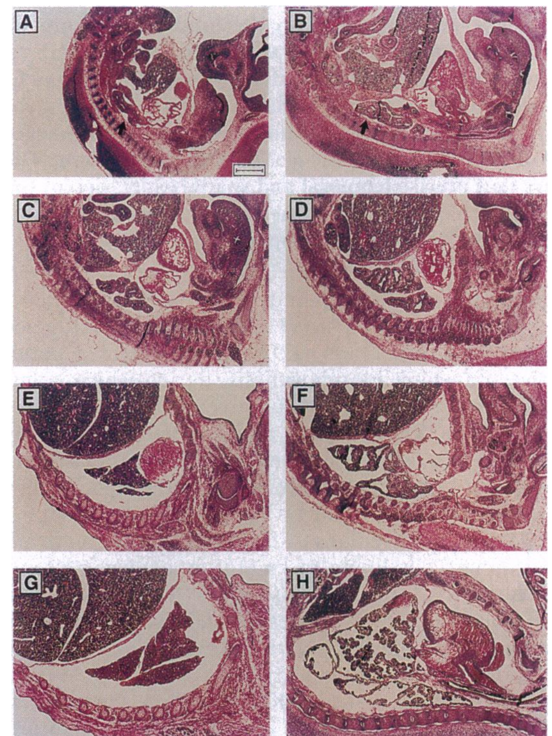

FIG. 1. Histology of lungs from gestational day-12.5 to -15.5 SP-C-hKGF transgenic and control nontransgenic littermate embryos. Saggital sections of nontransgenic control embryos (*A, C, E,* and *G*) and littermate SP-C-hKGF transgenic embryos (*B, D, F,* and *H*) were stained with hematoxylin and eosin and photographed under bright-field microscopy. Micrographs of sections from transgenic mice and control nontransgenic littermates are shown for gestational days 12.5 (*A* and *B*), 13.5 (*C* and *D*), 14.5 (*E* and *F*), and 15.5 (*G* and *H*). Enlarged luminal airways, resembling pulmonary cystadenoma, are observed in the developing transgenic lungs compared to littermate controls. Papillary cystadenomas of the lung were consistently observed in E15.5 embryos. Embryos shown are representative of several frozen embryos analyzed at each embryonic stage of development. Arrows point to lungs of the earliest stage embryos analyzed. (Bar = 500 μm.)

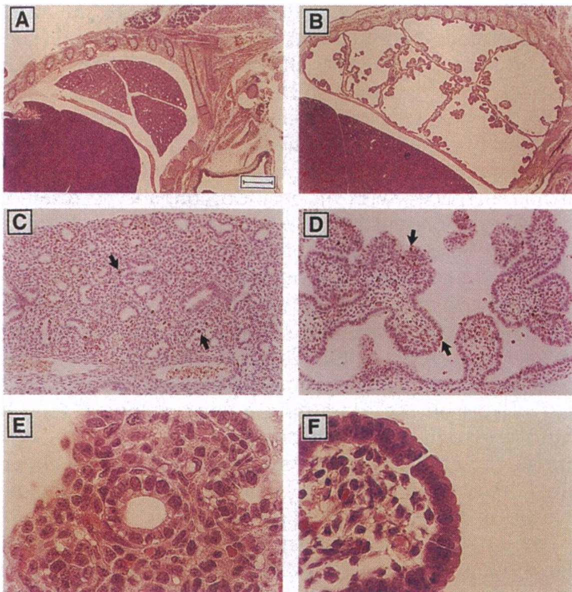

FIG. 2. Comparison of pulmonary development pattern and pulmonary epithelial proliferation and morphology in a normal mouse at gestational day 16.5 (E16.5) (*A, C,* and *E*) compared to a littermate SP-C-hKGF transgenic embryo (*B, D,* and *F*). (*A*) Photomicrograph of a saggital section showing lungs of a normal E16.5 mouse. (*B*) Photomicrograph of a saggital section showing lungs of an E16.5 SP-C-hKGF transgenic mouse lung. (*C*) Ki-67 staining of lungs from *A*. (*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*.)

of the SP-C-hKGF transgenic mice compared to their nontransgenic littermates (Figs. 1 and 2). During this period of embryonic development, the bronchial airways of normal mouse lungs are undergoing extensive branching, giving rise to secondary and tertiary airways. In several early stage transgenic embryos (E12.5–E14.5), the bronchial airways were enlarged, with the histology of the entire lung resembling a pulmonary cystadenoma. In more severe cases, papillary cystadenomas were observed. Bronchial airway enlargement was more evident in later stage embryos (E15.5–E16.5), where the lung morphology consistently resembled a papillary cystadenoma with large dilated saccules and numerous large airspaces (Figs. 1 and 2). At these later stages, there appeared to be a higher relative proportion of epithelium to mesenchymal tissue within the lungs of the transgenic mice compared to normal littermates. Normal branching morphogenesis appeared to be disrupted in the SP-C-hKGF transgenic embryos, and the cystic lungs occupied most of the thoracic cavity (Fig. 2 *A* and *B*). With the exception of this marked disruption of normal lung morphogenesis, all other organs and tissues of the SP-C-hKGF mice were morphologically normal. No signs of hyperproliferating squamous epithelial surfaces or increased epidermal thickness, indicative of systemic effects of KGF, were detected in tissues from any of the transgenic mice. In support of the *in vivo* effects of KGF on lung morphogenesis, cystic lesions and impaired branching morphogenesis were also observed in mouse fetal lung explants cultured in the presence of recombinant KGF (data not shown).

***In Situ* Hybridization for hKGF Localizes Transgene Expression Within the Developing Respiratory Epithelium.** To determine whether the morphological abnormalities in the developing lungs of different transgenic embryos correlate with expression of the transgene, *in situ* hybridization was performed to detect hKGF mRNA in saggital sections of transgenic and nontransgenic littermates (Fig. 3). hKGF mRNA was detected in epithelial cells lining the bronchial airways and papillary structures of transgenic mouse lungs. Although hKGF mRNA was detected in distal airway epithelium as well, distal airways were few and often difficult to locate in the transgenic mouse lungs. hKGF mRNA was expressed throughout the pulmonary epithelium of E12.5–E15.5 SP-C-hKGF transgenic embryos (Fig. 3 *B, D, F,* and *H*). *In situ* hybridization for endogenous mouse KGF receptor (KGFR) mRNA revealed that the receptor was expressed at all developmental stages in the same epithelial cells that expressed the transgene (data not shown). No evidence of KGFR mRNA upregulation was evident in any of the transgenic embryos. hKGF mRNA was not detected in other tissues or in the lungs of nontransgenic embryos analyzed at any stage of gestation (Fig. 3 *A, C, E,* and *G*). The intensity of the *in situ* hybridization signal for KGF mRNA correlated well with the severity of morphological abnormalities, being strongest in lungs exhibiting the most severe airway dilation and cystadenoma phenotype. Lower levels of hKGF mRNA were detected in the lungs of animals with more modest disruption of lung morphogenesis.

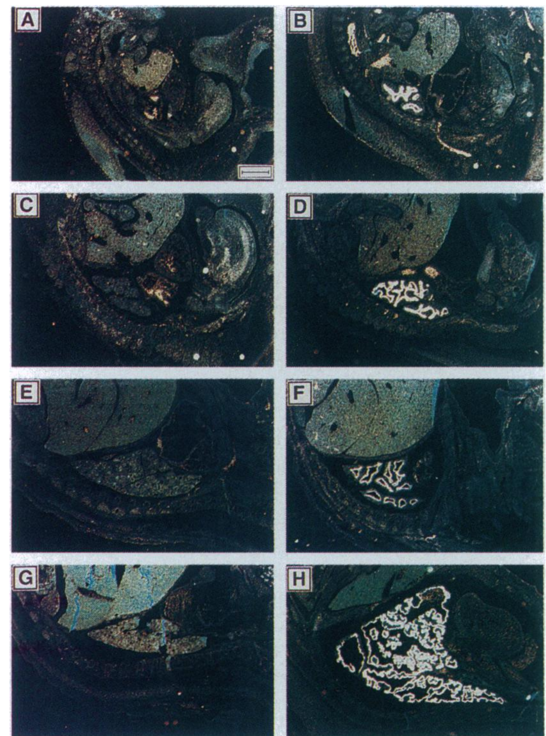

FIG. 3. *In situ* hybridization analysis of hKGF mRNA expression in developing lungs of gestational day-12.5 to -15.5 SP-C-hKGF transgenic and control mouse embryos. Saggital sections of nontransgenic control embryos (*A, C, E,* and *G*) and littermate SP-C-hKGF transgenic embryos (*B, D, F,* and *H*) were hybridized overnight with UTP[^{35}S]-labeled sense (data not shown) and antisense RNA probes for detection of hKGF mRNA. Dark-field micrographs of sections from transgenic mice and control nontransgenic littermates are shown for gestational days 12.5 (*A* and *B*), 13.5 (*C* and *D*), 14.5 (*E* and *F*), and 15.5 (*G* and *H*). Note the high levels of hKGF mRNA detected in the epithelium lining dilated and malformed airways (or bronchioles) of the developing lungs of transgenic embryos. Dilated bronchial tubules are evident at all time points. Note the somewhat milder dilation of the bronchial airways and lower hybridization signal in the gestational day-14.5 transgenic embryo (*F*). Results shown are representative of several embryos analyzed at each embryonic stage of development. (Bar = 500 μm.)

Table 2. Analysis of pulmonary epithelial markers in SP-C-hKGF transgenic and control mouse embryos

Epithelial marker	Control	SP-C-hKGF transgenics
SP-C mRNA	++	++
SP-C	+++	+
SP-B mRNA	+	+++
SP-B	−	−
Glycogen	+	+++
Mucin	−	−

Pulmonary epithelial cells were identified by morphology and confirmed by staining for thyroid transcription factor 1, a marker of pulmonary epithelium. proSP-C and proSP-B levels were assessed by immunostaining, SP-C and SP-B mRNA levels were assessed by *in situ* hybridization, and glycogen and mucin staining were assessed by periodic acid/Schiff reagent, with and without diastase, and mucicarmine staining, respectively. Each stain was graded as nondetectable (−), weak (+), strong (++), or very strong (+++).

Bronchial Epithelial Differentiation Is Compromised in the SP-C-hKGF Transgenic Mice. Lungs from transgenic and control embryos were stained for expression of Ki-67 (Fig. 2 *C* and *D*) and proliferating cell nuclear antigen (not shown), markers of cellular proliferation. Results from staining for both markers were similar and revealed that cellular proliferation was high in both transgenic and normal developing pulmonary epithelium. No obvious difference in the number of cells staining positive for these markers was evident within the transgenic and littermate control lungs.

Close analysis of the pulmonary epithelial morphology revealed that lungs from transgenic embryos (i.e., E15.5 and E16.5) were lined by columnar epithelial cells with oval nuclei (Fig. 2 *E* and *F*). The morphology of the respiratory epithelium was similar to that of primordial pulmonary epithelium and epithelium lining bronchial airways (2, 3). In contrast, epithelial cells within the distal airways of nontransgenic control mice were cuboidal with rounded nuclei, with morphologic features characteristic of more mature alveolar epithelium. Staining for expression of proSP-C, a marker for presumptive type II cells located in the distal acinar tubules and buds of the developing lung, was weak within the columnar epithelium of the SP-C-hKGF transgenic mouse lungs relative to the extensive staining of more distal airway epithelium of normal mouse lungs (Table 2). Furthermore, glycogen staining was abundant within the pulmonary epithelium of the transgenic mice relative to the lungs of nontransgenic littermates, where intense glycogen staining was limited to primitive airways. Histologic features of the SP-C-hKGF mouse lungs are consistent with disruption of normal fetal differentiation and maintenance of a nondifferentiating columnar epithelium characteristic of the embryonic lung. No ciliated cells were present, and all respiratory epithelial cells were negative for Clara cell secretory protein, a marker of bronchiolar cell differentiation (2). Staining for SP-B protein and mucicarmine was also negative in both transgenic and control lungs. In contrast to SP expression, SP-C and SP-B mRNA were detectable in the pulmonary epithelium of control and transgenic airway epithelium. SP-B mRNA expression in particular was much higher in epithelial cells of the transgenic lung relative to controls. The present findings demonstrate that KGF does not promote inherent maturation of the fetal respiratory epithelium. The SP-C-hKGF transgenic pulmonary epithelium may remain in an immature state, which is incapable of expressing SP, despite the presence of abundant surfactant message.

DISCUSSION

In the present study, transgenic mice were generated in which the expression of hKGF was directed to the epithelium of developing mouse lungs using the promoter of the human SP-C gene. These mice exhibited a striking embryonic lethal (E15.5–E17.5) phenotype with gross and histologic alterations in lung morphology prior to loss of viability. Ectopic expression of KGF in this manner resulted in an abnormal growth pattern of pulmonary epithelium during the pseudoglandular stage of lung development. There was a marked enlargement of the bronchial airspaces, with the resulting phenotype resembling papillary cystadenoma. The lungs exhibited exaggerated large airways, a deficiency of small branching airways, and a relative paucity of mesenchyme at a time during embryonic development when normal lungs display abundant mesenchymal tissue and branching morphogenesis. The airway epithelium showed evidence of incomplete differentiation compared to epithelium of age-matched normal embryos. Recombinant KGF had similar effects on the gross morphology of mouse fetal lung explants grown in tissue culture.

During the early phases of normal lung development (preceding E14.5), the epithelial cells of the primordial tubules are columnar in shape and contain abundant glycogen (1–3). Between E14.5 and E16.5, the primitive respiratory tubules, or acinar tubules, are formed in the periphery and contain low columnar or cuboidal cells. The low columnar cells will become the terminal bronchioles and the cuboidal cells will become alveolar cells (1, 2). The columnar appearance of epithelial cells in the large dilated saccules of the SP-C-hKGF transgenic lungs suggests that the proliferating pulmonary epithelium may remain in a less differentiated or primitive stage of development characteristic of primordial or bronchial epithelium. In support of this observation, staining for proSP-C was weaker in columnar epithelium of the SP-C-hKGF transgenics than in more cuboidal distal airway epithelium of normal lungs, although there was some variability among different transgenic embryos. While SP-B mRNA levels were markedly elevated in the transgenic epithelium, immunoreactive proSP-B was undetectable, perhaps reflecting maintenance of the cells in an undifferentiated state where SP expression is low, glycogen is abundant, and the cells are columnar shaped. KGF has been demonstrated to increase SP-B mRNA expression in cultured alveolar type II cells (23).

Administration of hKGF to adult rat lungs has been demonstrated to stimulate proliferation and differentiation of cuboidal type II pneumocytes (13), supporting previous results indicating that administration of KGF to intact animals can stimulate epithelial cell growth as well as influence differentiation of epithelial cells (24–26). In these transgenic animals, the continuous unregulated presence of KGF appears to have impaired normal epithelial differentiation and lung morphogenesis at a stage in embryonic lung development when alveolar airways have yet to develop. Preliminary observations in transgenic mice engineered to express KGF in the liver of late-gestation embryos (E18.5 and E19.5) reveal type II cell proliferation in the lungs (presumably due to systemic KGF). Thus, while KGF did not stimulate differentiation of bronchial epithelium into alveolar epithelium in these transgenic mice, KGF has been demonstrated in other experimental systems to stimulate existing type II alveolar cells to proliferate.

In a recently published study, targeted expression of a dominant-negative FGFR2-IIIb splice variant to the developing pulmonary epithelium, using the same promoter as in the work presented here, completely blocked normal mouse lung branching morphogenesis and epithelial differentiation (14). The mice died from respiratory failure immediately after birth. Those results demonstrated the importance of this receptor in normal pulmonary development and by implication the importance of one or more of the members of the FGF family that interact with the receptor. The results of the present study demonstrate an important role for one such member of the FGF family, specifically KGF. The observed phenotypes of the transgenic mice in the two studies are consistent: inhibition of the signaling pathway by blocking the KGF receptor yields

underdeveloped lung epithelial structures, whereas superstimulation of the pathway by KGF overexpression results in overabundance of columnar epithelium and greatly enlarged airways.

The results of the present study lend support for a critical role of mesenchyme-derived KGF in mediating aspects of normal lung epithelial growth and maturation. Supporting this conclusion is the observation that KGF and its receptor are expressed in normal adult rat lungs (13). In addition, KGF mRNA has been localized in embryonic lung mesenchyme (12), and FGFR2, the gene encoding the KGF receptor (27), is expressed in airway epithelium from the earliest stages of lung bud formation through late fetal lung development (28, 29). Recent findings demonstrate that KGF expression is upregulated in the mesenchyme of human fetal lung explants as they mature in culture over a period of several days (S. Dekowski, W.S.S., and R. Panos, unpublished data). Thus, a growing body of evidence supports a role for mesenchyme-derived KGF in mediating normal lung development. When KGF expression is uncoupled from normal mesenchymal cell regulation, as in the SP-C-hKGF transgenic mice, normal growth and differentiation signals are disrupted.

The large cystic lesions are histologically similar to lungs of premature and full-term human infants with congenital cystic adenomatoid malformations (30). Affected regions of the lungs of individuals with cystadenomatoid malformations are characterized by the presence of abnormal cystic structures lined by cuboidal, columnar, or pseudostratified columnar bronchiolar-like epithelium. The histological similarities to the lungs of the SP-C-hKGF transgenic mice raise the possibility that disruption of KGF-dependent interactions between the epithelium and mesenchyme may be involved in the pathogenesis of cystadenomatoid malformations seen in humans.

The embryonic lethality that accompanied overexpression of KGF in the lung was unexpected in light of previous observations that genetic manipulations that grossly affect lung development (15, 16, 18, 31) or completely ablate normal branching morphogenesis of the developing lung (14) have not resulted in prenatal lethality. Because normal lung function is not a prenatal requirement, death of the SP-C-hKGF transgenic embryos is likely a consequence of the cystadenoma-like morphology observed in E15.5 and E16.5 KGF transgenic embryos, where the lung occupies virtually the entire thoracic cavity and may have impaired cardiac function and blood circulation. KGF may exert effects on physiological systems (i.e., atrial natriuretic factor, angiotensin converting enzyme, or ion transporters) that influence normal cardiac function. While it is possible that systemic KGF may contribute to embryonic lethality, no systemic effects (i.e., abnormal skin appearance or hair follicle development, enlarged organs, or general thickening of squamous epithelial surfaces), which are noted when recombinant KGF is injected into rodents (24, 25), were evident.

The results of the present study suggest that KGF is a growth factor with potent effects on the developing morphology of the embryonic lung and, in its normal context, acts as an important stromal-derived mediator of branching morphogenesis.

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