Fibroblast Growth Factor 18 Influences Proximal Programming during Lung Morphogenesis*

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The structure and functions of the airways of the lung change dramatically along their lengths. Large-diameter conducting airways are supported by cartilaginous rings and smooth muscle tissue and are lined by ciliated and secretory epithelial cells that are involved in mucociliary clearance. Smaller peripheral airways formed during branching morphogenesis are lined by cuboidal and squamous cells that facilitate gas exchange to a network of fine capillaries. The factors that mediate formation of these changing cell types and structures along the length of the airways are unknown. We report here that conditional expression of fibroblast growth factor (FGF)-18 in epithelial cells of the developing lung caused the airway to adopt structural features of proximal airways. Peripheral lung tubules were markedly diminished in numbers, whereas the size and extent of conducting airways were increased. Abnormal smooth muscle and cartilage were found in the walls of expanded distal airways, which were accompanied by atypically large pulmonary blood vessels. Expression of proteins normally expressed in peripheral lung tubules, including SP-B and pro-SP-C, was inhibited. FGF-18 mRNA was detected in normal mouse lung in stromal cells surrounding proximal airway cartilage and in peripheral lung mesenchyme. Effects were unique to FGF-18 because expression of other members of the FGF family had different consequences. These data show that FGF-18 is capable of enhancing proximal and inhibiting peripheral programs during lung morphogenesis.

The lung bud evaginates from the foregut endoderm and undergoes stereotypic dichotomous branching as it invades the splanchnic mesenchyme. With advancing development, the proximal conducting airways are distinguished from the lung periphery, which contains highly vascularized alveoli that mediate gas exchange. In the proximal lung, conducting airways are supported by cartilage and smooth muscle and lined by a stratified, pseudostratified, or simple columnar epithelium. In the normal lung periphery, alveolar ducts and alveoli are devoid of cartilage and lined by cuboidal and squamous cells. The mechanisms by which these proximal and distal regions of the respiratory tract are formed from endodermal precursors remain poorly understood.

Stereotypic branching of the endodermally derived lung buds requires inductive signals provided by the mesenchyme that are mediated, in part, by the binding of members of the fibroblast growth factor (FGF) family of polypeptides to fibroblast growth factor receptors (FGFRs) on target cells (1–3). Both in vivo and in vitro experiments support the critical role of FGF signaling in lung morphogenesis. FGF polypeptides, including FGF-1, FGF-2, FGF-7, FGF-9, FGF-10, and FGF-18, are expressed in the developing lung (4–11), as are FGF receptors FGFR1, FGFR2, FGFR3, and FGFR4 (12–16). The FGFR2-IIIb splice variant is expressed at high concentrations in the epithelial cells of the lungs buds (4), likely binding FGF family members that are secreted locally to regulate cellular activities in a paracrine manner. Because the sites and levels of expression in FGF family members and receptors vary during lung development, the precise temporal-spatial expression of various FGF polypeptides might influence the differentiation, proliferation, and migration of target cells, which in turn might influence the formation of the distinct proximal and peripheral regions of the lung.

The importance of FGF signaling in lung morphogenesis has been demonstrated in a number of animal models in vivo. Expression of a dominant-negative FGFR with the SP-C promoter in vivo blocked branching morphogenesis of the lung and was associated with complete loss of the distal subset of respiratory epithelial cells, demonstrating a critical requirement for FGFR signaling in lung formation (17). Likewise, selected targeting of the FGFR2-IIIb isoform blocked limb and lung development in vivo, findings identical to those in the FGF-10-null mice, supporting the primary role of FGFR signaling in lung morphogenesis (18). Whereas FGFR3- and FGFR4-null mice did not have abnormalities in lung formation, double-null FGFR3/FGFR4 mice developed emphysema in the postnatal period, demonstrating a more subtle effect of FGFR3 and FGFR4 on postnatal lung architecture (19). The precise ligands mediating FGF signaling by these receptors have not been clarified, and it remains unclear how the precise temporal, spatial, and stoichiometric expression of various FGF polypeptides might influence cell proliferation, migration, or differentiation of various lung cell types during normal branching morphogenesis. A model in which interactions between FGF-10 and...
and BMP-4 signaling pathways may regulate the sites and extent of proximal-distal lung maturation and branching has been proposed (20).

Whereas ectopic expression of FGF-7 in the developing lung in vivo or application of FGF-7 to lung explants in vitro disrupted branching morphogenesis and caused cystic malformations in the fetal lung (21, 22), neither FGF-7 nor FGF-10 altered the spatial patterning of respiratory epithelial cell markers or the proximal-distal patterning of lung structures in analogous mouse models. Postnatally, intratracheal administration of FGF-7 caused diffuse alveolar and bronchiolar cell hyperplasia and markedly increased the expression of surfactant proteins in type II epithelial cells, demonstrating the sensitivity of the postnatal lung to increased FGF signaling (23). Despite the marked proliferative effects of FGF-7 in vivo, morphogenesis of the fetal lung was not perturbed in FGF-7 gene-inactivated mice, suggesting redundant activity of FGF polypeptides or lack of a requirement for FGF-7 in lung formation (24). In contrast, deletion of FGF-10 blocked formation of the lung, with residual tissue consisting of only an upper trachea (25, 26). Effects of increased expression of FGF-10 on lung morphogenesis were similar to but also distinct from those of FGF-7; the former caused organized pulmonary adenomas in the postnatal period (27, 28). Conditional expression of both FGF-7 and FGF-10 caused proliferation of cuboidal type II epithelial cells and increased the expression of type II epithelial cell markers including pro-SP-C and SP-B in the fetal lung and did not perturb proximal to distal patterning of the conducting airways (27, 28). Recent evidence also supports a distinct role for FGF-9 in lung morphogenesis. FGF-9 is expressed most highly in the epithelium and visceral pleura of the lung, and its targeted deletion causes lung hypoplasia in vivo (6). Because many FGF family members are expressed in the developing lung, the precise roles of each of these polypeptides and whether they serve distinct or overlapping functions in the formation of the lung remain unclear.

FGF-18 is a member of the FGF polypeptide family expressed in various tissues including the lung. In the fetal lung, FGF-18 was detected in subsets of mesenchymal cells; the timing and pattern of expression of FGF-18 were distinct from those of FGF-10 or FGF-9 (8). FGF-18 binds to various FGFRs with high affinity but does not bind to FGFR1 isoforms, properties that overlap with those of FGF-7 (which binds and activates FGFR2-IIIb) and FGF-10 (which binds to both FGFR2-IIIb and FGFR1-IIIb receptor isoforms) (29, 30).

To assess the role of FGF-18 during lung morphogenesis, conditional expression of FGF-18 was achieved in transgenic mice utilizing the reverse tetracycline transactivator (rtTA) in respiratory epithelial cells under control of the SP-C promoter. In contrast to findings in similar models expressing FGF-7 or FGF-10, FGF-18 altered the length, caliber, and epithelial cell differentiation of conducting airways, increasing the size of peripheral pulmonary blood vessels. In addition, FGF-18 induced ectopic cartilage formation in the lung, supporting the concept that FGF-18 selectively influenced the programming of a number of proximal elements of the lung at the expense of peripheral lung development.

**EXPERIMENTAL PROCEDURES**

Transgenic Mice—A permanent transgenic mouse line bearing the SP-C-rtTA transgene was established in FVB/N background after oocyte injection of a plasmid construct consisting of 3.7 kb of the human SP-C promoter placed 5’ to the rtTA gene construct (27, 28, 31). The mouse FGF-18 cDNA was inserted between the (teto)CMV-bGH-poly(A) vector, and the resulting transgenic mice were bred to SP-C-rtTA mice. Heterozygous and homozygous transgene (lines A and B) were chosen for breeding to SP-C-rtTA mice. Transgene expression was confirmed by northern blotting using an FGF-18 cDNA probe. From those mice, offspring were also mated to produce double transgenic mice that were homozygous or heterozygous for each transgene, transmitting the (teto)CMV-FGF-18 transgene to SP-C-rtTA mice. The transgenic SP-C-rtTA “activator” line was used in all experiments.

To produce a model of increased FGF-18 expression in the fetal lung, (teto)7CMV-FGF-18 mice were viable and without observable abnormalities. Two separate target lines bearing the (teto)CMV-FGF-18 transgene lines A and B were chosen for breeding to SP-C-rtTA mice. Transmission of both transgenes followed typical Mendelian inheritance patterns. All mice were maintained in a pathogen-free vivarium. Doxycycline (0.5 mg/ml) was administered in drinking water or in food pellets (25 mg/g; Harlan Teklad, Madison, WI) for the described time periods. Drinking solution containing doxycycline was changed 3 times/week, whereas activity of the doxycycline was stabilized in the food pellet (33).

Reverse Transcription-PCR—Tissues were homogenized in Trizol (Invitrogen), and RNA was isolated according to the manufacturer’s specifications. DNA was treated with DNase before cDNA synthesis. Five µg of RNA was reverse-transcribed and then analyzed by PCR for murine FGF-18 and the transgene-specific FGF-18 and β-actin mRNAs. Transgene-specific primers for mouse FGF-18 were designed to the (teto)CMV-FGF-18 transcript and used for amplification. Primer A was located in the CMV minimal promoter (5’ to 3’): AGAGGCAATC-ACGCTTGTGG; primer B was located within the FGF-18 cDNA (5’ to 3’): CAGGACTTGAATGTGCTTCCCACTG. FGF-18 mRNA was compared with that amplified for β-actin. FGF-18 mRNAs were also estimated using primers designed to amplify within the FGF-18 coding sequence using standard gel analysis of PCR products. FGF-18, FGF-10, sonic hedgehog, BMP-4, and Sprouty-2 mRNAs were also determined by real-time PCR of lung cDNA, after optimization of primers and conditions. Dams were placed on doxycycline throughout pregnancy and sacrificed on embryonic day 20. RNA was extracted from the lungs of each pup. cDNA was prepared by reverse transcription and analyzed on the Smart Cycler® using primers to identify β-actin, FGF-18, BMP-4, Sprouty-2, and FGF-10. All results were normalized to β-actin.

**Histology, Immunohistochemistry, and Electron Microscopy**—To obtain fetal lung tissue, the fetuses were removed by hysterectomy after lethal injection of pentobarbital to the dam. The chest of fetal animals was opened, and the tissue was fixed with 4% paraformaldehyde at 4 °C. Lungs from postnatal animals were inflation-fixed at 25 cm water pressure via a tracheal cannula with the same fixative. Tissue was fixed overnight, washed in phosphate-buffered saline, dehydrated through a series of alcohols, and embedded in paraffin. Tissue sections were stained for SP-B, pro-SP-B, TTF-1, pro-SP-C, Clara cell secretory protein (CCSP), peripheral endothelial cell adhesion molecules (PECAMs), α-smooth muscle actin, Foxj1, and pro-collagen II using methods described previously (27, 28). Cartilage was stained with Alcian blue, and

**Fig. 1. Transgenic constructs used for conditional expression of FGF-18.** Mouse FGF-18 cDNA was inserted into the (teto)CMV-bGH-poly(A) vector, and the resulting transgenic mice were bred to SP-C-rtTA mice. The transgenic SP-C-rtTA “activator” line used has been stable for more than 3 years in the vivarium. Heterozygous and homozygous (teto)CMV-FGF-18 mice were viable and without observable abnormalities. Two separate target lines bearing the (teto)CMV-FGF-18 transgene lines A and B were chosen for breeding to SP-C-rtTA mice. Transmission of both transgenes followed typical Mendelian inheritance patterns. All mice were maintained in a pathogen-free vivarium. Doxycycline (0.5 mg/ml) was administered in drinking water or in food pellets (25 mg/g; Harlan Teklad, Madison, WI) for the described time periods. Drinking solution containing doxycycline was changed 3 times/week, whereas activity of the doxycycline was stable in the food pellet (33).
residual tissue was dissolved in KOH before photography. For electron microscopy, tissue was fixed, prepared, and evaluated as described previously (27).

**In Situ Hybridization—**Expression of mouse FGF-18 mRNA was assessed by **in situ** hybridization using 35S-labeled riboprobes as described previously for fetal and adult lungs (27); the latter were assessed after inflation fixation at 25 cm of water pressure. Sense and antisense FGF-18 RNA probes were generated in PGEM32. Tissue was hybridized overnight at 50 °C. Slides were coated with Kodak NTB2 emulsion, exposed for 7–14 days, and developed with Kodak D19. Whole mount **in situ** hybridization for mouse FGF-18, FGF-10, sonic hedgehog, BMP-4, and Sprouty-2 was performed by digoxigenin-labeled cDNA antisense and sense probes. Whole mount **in situ** hybridization was carried out on the lungs of fetal day 12 embryos, whose dams had been on doxycycline throughout pregnancy. Antisense and sense probes were made from transcription vectors, using digoxigenin-UTP as label. After hybridization and washing, anti-digoxigenin antibody coupled to alkaline phosphatase substrate was adsorbed. The product was developed using BM purple alkaline phosphatase substrate.

**RESULTS**

**Generation of SP-C-rtTA and (teto)7CMV-FGF-18 Transgenic Mice—**In the absence of doxycycline, double transgenic SP-C-rtTA and (teto)7CMV-FGF-18 mice (heterozygous for each transgene) were viable. Fetal and postnatal single transgenic mice were produced in ratios predicted by Mendelian inheritance. Lung morphology was normal in both single transgenic mice and double transgenic mice in the absence of doxycycline. Previous **in situ** hybridization and reporter gene analyses of the lungs from SP-C-CAT and SP-C-rtTA mice demonstrated that transgenic mRNA was selectively expressed in peripheral respiratory epithelial cells in the lungs of fetal and adult mice. Expression of firefly luciferase with the SP-C-rtTA system was observed as early as E10 in vivo (33). In adult SP-C-rtTA mice, rtTA mRNA was selectively expressed in peripheral conducting airways and type II epithelial cells (28, 33). Two independent lines of (teto)7CMV-FGF-18 target mice (lines A and B) were generated and mated to SP-C-rtTA mice. A similar morphologic phenotype was observed in the lungs of the independent (teto)7CMV-FGF-18 lines after exposure to doxycycline. Subsequent studies utilized (teto)7CMV-FGF-18 line A.

**Conditional Expression of FGF-18 mRNA—**Transgene-specific FGF-18 mRNA was assessed by reverse transcription-PCR in the lungs of young adult mice with and without the addition of 0.5 mg/ml doxycycline in the drinking water. In adult double transgenic SP-C-rtTA × (teto)7CMV-FGF-18 mice, FGF-18 mRNA was detectable at low levels in the absence of doxycycline, representing some “leak” in the absence of doxycycline, but was induced after oral doxycycline as described previously with the SP-C-rtTA mice (27, 28). Exposure of adult double transgenic mice to doxycycline did not alter lung morphology (data not shown). In pups obtained from dams treated with doxycycline, FGF-18 mRNA was detected in fetal SP-C-rtTA × (teto)7CMV-FGF-18 double transgenic mice but was not readily detected in single transgenic animals (Fig. 2). Transgenic FGF-18 mRNA was not detected in other major organs of double transgenic mice, including liver, spleen, kidney, and brain, typical of the specificity of the SP-C promoter element (31), which is generally active including liver, spleen, kidney, and brain, typical of the specificity of the SP-C promoter element (31), which is generally active only in respiratory epithelial cells in the lung (data not shown). Exogenous FGF-18 mRNA was detected in the testes of a double transgenic mouse on doxycycline, albeit at extremely low levels compared with that seen in the lung.

**Effects of FGF-18 on the Fetal Lung—**When dams were exposed to doxycycline on E6 and maintained on doxycycline during pregnancy, the percentage survival of the offspring decreased to 50% of the expected Mendelian numbers in double transgenic mice, consistent with the lethality of the transgene. The structure of lungs from double transgenic SP-C-rtTA × (teto)7CMV-FGF-18 offspring was assessed at E16–E19 (Fig. 3). Without doxycycline, lung histology of double transgenic mice was generally indistinguishable from normal. In the presence of doxycycline, dramatic histological abnormalities were observed in the lungs from double transgenic mice on E16–E19. Branching morphogenesis was disrupted. A marked increase in the length and caliber of conducting airways was observed. Decreased branching of peripheral airways with a marked reduction in peripheral sacculi was consistently noted. The abnormal histology was not observed on E12.5 but was readily apparent at E16 and thereaf-
and control littermates. Lungs were dissected on E17 from double expressing mice. Photographed under a dissecting microscope. Abnormal airways and marked dilation of peripheral lung saccules were observed in FGF-18-expressing mice. Arrows indicate dilated saccules.

Later in gestation, on E18 and E19, stage-specific sacculation of peripheral acinar buds and alveoli were lacking in the FGF-18-expressing mice (Fig. 3). Elongated conducting airways of disordered caliber extended to the lung periphery. Atypical branching of bronchioles and distal bronchial-acinar tubules was observed, and normal acinar ducts and alveoli were markedly decreased or absent. The lung mesenchyme was thickened, containing few acinar tubules. Pulmonary blood vessels were prominent, with abnormally large lumens, and alveolar capillaries were lacking. The residual peripheral airway saccules were not dilated, and the normal alveolar structures of the newborn lung were lacking. Large, abnormal bronchial-like tubules were observed throughout the lungs, and many extended to the pleural surfaces. Whereas similar abnormalities were observed in FGF-18-expressing fetal mice, variability in the extent of the histological abnormalities was observed even in double transgenic mice from the same litter, suggesting that the timing, extent, or levels of transgene expression may influence the severity of the phenotype. Similar lung abnormalities were observed from litters capable of producing offspring with one copy or two copies of the CMV-FGF-18 transgene and in FGF-18-expressing mice. Abnormal pro-SP-C and SP-B staining were also observed on E19 (H). CCSP, a marker of conducting airway epithelial cells, was detected in a contiguous cluster of cells lining the abnormal tubule (J) (original magnification, ×6).

Aberrant Morphology and Differentiation of Epithelial Cells Lining the Lung Tubules—The peripheral conducting tubules in FGF-18-expressing mice at E16–E19 were lined by a relatively homogenous population of columnar and cuboidal epithelial cell cilia. These abnormal airway epithelial cells stained intensely and homogeneously for TTF-1, reflecting a lack of terminal differentiation and failure to form squamous cells (type I) in the periphery at E16–E19 and in newborns (Fig. 5 and data not shown). Pro-SP-C and SP-B were detected at relatively low levels throughout the abnormal epithelium, consistent with the lack of both type II and squamous type I cell differentiation at E16 and E19. Atypical staining of surfactant proteins was observed in both basal and apical regions of the cells, whereas staining was detected in the apical regions of type II cells in the normal lung. Abnormal clumps of cells staining for CCSP were observed in the elongated dilated respiratory bronchioles. However, CCSP was excluded from the most peripheral regions of lung tubules at E16 and E19 (Fig. 5 and data not shown), as it is in the alveolar regions of the normal lung. The atypical cells lining lung tubules did not express FoxJ1, a marker of ciliated cells in normal conducting airways (data not shown).

FGF-18 Altered Differentiation and Morphology of the Pulmonary Mesenchyme—Pulmonary vascular development was perturbed as indicated by the abnormalities of PECAM staining in the pulmonary mesenchyme of the FGF-18-expressing mice (Fig. 6). Extensive blood vessel development was noted in the abnormal mesenchyme surrounding the sparse, relatively small acinar tubules. Atypical pulmonary blood vessels in the periphery often had a markedly enlarged lumenal diameter (Fig. 6). α-Smooth muscle actin (α-SMA) staining, which is normally abundant in proximal conducting airways and excluded from the alveolar region, was observed surrounding the aberrant airways in the lung periphery and detected at sites that normally lack α-SMA staining in control littermates (Fig. 6).

Ultrastructure of Fetal Lung from FGF-18-expressing Mice—Ultrastructural analysis of lung tissue from FGF-18-expressing mice at E16 and E18 (the latter is not shown) was consistent with observations at the light microscopic level.
Abnormally large peripheral airways were lined by an atypical columnar epithelium. A relatively homogenous population of immature epithelial cells was observed in the peripheral tubules. Most of the terminal airspaces were lined by cuboidal or columnar cells, and few squamous (type I) cells were observed. Epithelial cells were rich in glycogen, often lacked microvilli, and contained few lipid inclusions (Fig. 7). Tubular myelin was not observed in the airways. Some of the atypical cells contained basal bodies, which are typical of developing tracheal-bronchial ciliated cells. In lungs from control littersmates, developing pre-type II cells were cuboidal and contained putative lamellar bodies. Abnormal epithelial cells had a homogenous morphology, were rich in glycogen, lacked extensive microvilli, and contained few lamellar bodies and other intracellular organelles. Basal bodies were seen in some of the atypical cells.

BMP-4, Sprouty-2, and FGF-10 mRNAs confirmed the lack of effect of FGF-18 on these mRNAs.

In Situ Hybridization for Endogenous FGF-18 mRNA—In situ hybridization with radiolabeled mouse FGF-18 antisense RNA demonstrated that FGF-18 mRNA was expressed at high concentrations in stromal cells surrounding the tracheal-bronchial cartilage rings, in tissue surrounding the laryngeal cartilage, and in the mesenchyme of normal fetal lung from E12.5–E18 (Fig. 10 and data not shown).

Temporal Requirement for Disruption of Lung Morphology by FGF-18—When assessed on E18 to E18.5, lung morphology was severely disrupted in pups from dams treated with doxycycline from E3–E11. Taken together, these experiments define the period from E11–E14 as critical for generation of the severe lung abnormalities seen in FGF-18-expressing mice.

DISCUSSION

FGF-18 mRNA was conditionally expressed in respiratory epithelial cells of the lungs of fetal and postnatal mice. FGF-18 had little effect on the postnatal lung. However, lung morphogenesis was perturbed by expression of FGF-18 in the fetal lung. FGF-18 increased the length and caliber of peripheral conducting airways, disrupted branching of peripheral conducting airways, and altered cytodifferentiation of epithelial cells lining the bronchial-like lung tubules. In addition, FGF-18
Effects of FGF-18 on Lung Structure

FGF-18 Influences Lung Morphogenesis

FIG. 9. Histology and collagen II staining of abnormal cartilage. Hematoxylin and eosin staining demonstrated cartilage rings in wild-type (A) and FGF-18-expressing mice at E16 (B). Collagen II staining of sections of the fetal lung from FGF-18-expressing (C) and control (D) mice is shown at E16. Altered morphology of tracheal cartilage, expansion of cartilage rings in the bronchiolar region, and extensive bronchial cartilage that stained intensely for collagen II in the lung periphery were observed. Original magnification, ×4.

FIG. 10. In situ hybridization analysis of endogenous FGF-18 mRNA. In situ hybridization was performed with radiolabeled FGF-18 antisense (A and B) and sense probes (B and C) on sections of fetal mouse tissue from wild-type mice on E18.5 (top panels). Dark-field analysis demonstrated FGF-18 mRNA surrounding cartilage-forming zones in the larynx and tracheal rings and diffusely in the mesenchyme of fetal lung parenchyma. A similar pattern of expression was observed on E16 (data not shown). Original magnification, ×4.

Effects of FGF-18 on Lung Structure—Abnormalities in the FGF-18-expressing mice were quite distinct from those induced by FGF-7 and FGF-10 (27, 28); the latter peptide caused generalized epithelial cell hyperplasia and/or cyst formation in the lung periphery. Increased expression of FGF-7 and FGF-10 in similar transgenic models selectively increased the number of type II cells and increased expression of TTF-1, pro-SP-C, and SP-B. In contrast, FGF-18 produced a homogenous cuboidal-columnar epithelium that lacked features characteristic of normal, peripheral tubules. The atypical columnar epithelial cells were rich in glycogen and lacked other features typical of type II cells. Squamous cell differentiation was inhibited. On the other hand, some aspects of proximal epithelial cell differentiation were not apparent in the abnormal epithelial cells. Neither cilia, Foxj1, nor CCSP staining was observed in most of the atypical epithelial cells in the peripheral lesions induced by FGF-18. Likewise, FGF-18 did not alter the levels or sites of expression of FGF-10, BMP-4, and Sprouty-2 mRNAs, suggesting that the effects of FGF-18 on lung morphology were not mediated via these pathways.

Effects of FGF-18 were distinct from those of FGF-7 and FGF-10 when expressed with the SP-C promoter in vivo. In contrast to FGF-18, FGF-7 and FGF-10 increased the expression of type II cell markers SP-C and SP-B (27, 28). In vitro, FGF-7 and FGF-10 increased proliferation of type II cells and bronchiolar cells, enhancing the expression of SP-C (21, 23, 34, 35). The sites and levels of expression of the FGF family members vary in developing lung, and each FGF family member is capable of binding or interacting with various FGF receptors. A number of extracellular and intracellular mechanisms further influence FGF signaling. Effects of FGFs on the fetal lung may be altered by factors that limit synthesis and secretion of FGF ligands, or modulate receptor binding and intracellular signaling (36). It is also unclear whether various FGF ligands may compete for binding sites or receptors. The sites and levels of ectopic expression of FGF-18 may have influenced the observed morphological effects of the FGF-18 transgene. In the present study, FGF-18 was expressed in epithelial cells and not in mesenchymal cells, as in wild-type mice; therefore, bioavailability of the ligand or accessibility of the ligands to FGF receptors may be distinct in the transgenic mice. In situ hybridization for endogenous FGF-18 mRNA confirmed its expression in the pulmonary mesenchyme and demonstrated its distribution surrounding forming cartilage rings in the trachea and bronchi. This site of expression is consistent with a potential role for FGF-18 in cartilage formation.

Increased Cartilage Formation—Expression of FGF-18 perturbed cartilage ring morphology in the trachea and expanded cartilaginous tissue in the peripheral regions of main bronchi. The presence of endogenous FGF-18 mRNA surrounding normal cartilage rings in the developing trachea is also consistent with the role for FGF-18 and FGFR signaling in tracheobronchial cartilage morphogenesis. Ectopic cartilage was not seen on E12.5 but was readily apparent at E16. Abnormalities in cartilage and lung parenchyma were not observed when FGF-18 was expressed postnatally (data not shown), and exposure to doxycycline from E11 to E14 was required to generate the phenotype. The shape and contiguity of cartilaginous rings were perturbed at normal sites of tracheal-bronchial cartilage formation; however, large amounts of cartilage formed in the distal bronchi of the FGF-18-expressing mice. Chondrocyte proliferation and differentiation are strongly influenced by FGF receptor signaling, FGFR1 and FGFR3 have distinct and opposing roles in chondrocyte development in vitro and in vivo (37). Of considerable clinical interest, similar abnormalities in tracheal ring morphology are associated with Apert’s syndrome, a genetic disorder associated with craniofacial and hand malformations. Apert’s syndrome is caused by activating mutations in the FGFR2 gene, one of the FGF receptors activated by FGF-18 (38). FGFR3 is expressed in resting cartilage, and disruption of FGF signaling by Sprouty disrupts morphogene-
sis of bone and cartilage in vivo (39, 40). In the transgenic mice, abnormalities in cartilage formation were most pronounced in the peripheral bronchi-proximal bronchiolar, likely reflecting the activity of SP-C-rTA, which is more active in the bronchiolar and alveolar regions of the lung (41). However, disruption of more proximal tracheal ring morphology was apparent, suggesting that the transgene was active early in development, during which the surfactant protein promoter is expressed in the distal regions of the conducting airways. These findings support the concept that FGF-18 altered proliferation of chondrocytes or prechondrocytes at critical times during morphogenesis. The abnormal cartilaginous tissue stained intensely for collagen type II, an early marker of cartilage differentiation. Thus, changes in epithelial differentiation did not represent a complete proximalization of the peripheral bronchi-proximal bronchiolar, likely reflecting development and morphogenetic programs of blood vessels, cartilage, and airways toward proximal programs.

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