FGF-10 disrupts lung morphogenesis and causes pulmonary adenomas in vivo

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Clark, Jean C., Jay W. Tichelaar, Susan E. Wert, Nobuyuki Itoh, Anne-Karina T. Perl, Mildred T. Stahlman, and Jeffrey A. Whitsett. FGF-10 disrupts lung morphogenesis and causes pulmonary adenomas in vivo. Am J Physiol Lung Cell Mol Physiol 280: L705–L715, 2001.—Transgenic mice in which fibroblast growth factor (FGF)-10 was expressed in the lungs of fetal and postnatal mice were generated with a doxycycline-inducible system controlled by surfactant protein (SP) C or Clara cell secretory protein (CCSP) promoter elements. Expression of FGF-10 mRNA in the fetal lung caused adenomatous malformations, perturbed branching morphogenesis, and caused respiratory failure at birth. When expressed after birth, FGF-10 caused multifocal pulmonary tumors. FGF-10-induced tumors were highly differentiated papillary and lepidic pulmonary adenomas. Epithelial cells lining the tumors stained intensely for thyroid transcription factor (TTF)-1 and SP-C but not CCSP, indicating that FGF-10 enhanced differentiation of cells to a peripheral alveolar type II cell phenotype. Withdrawal from doxycycline caused rapid loss of the differentiation markers TTF-1, SP-B, and proSP-C, FGF-10 disrupted lung morphogenesis and induced multifocal pulmonary tumors in vivo and caused reversible type II cell differentiation of the respiratory epithelium.

fibroblast growth factor; conditional expression; epithelial cell differentiation; adenoma; respiratory failure; lung bud; fetal lung

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tides or lack of a requirement for FGF-7 in lung formation (12). FGF-1, FGF-2, FGF-9, FGF-10, and FGF-18 are also expressed in the developing lung. The precise roles of each of these polypeptides and whether they serve distinct or overlapping functions in lung morphogenesis or repair are not known at present.

Most FGF polypeptides bind with overlapping specificity to FGFR. FGF-7 is unique in binding selectively to FGFR2-IIIb; FGF-10, however, binds and activates both FGFR2-IIIb and FGFR1-IIIb in vitro (22). Expression of an FGFR dominant negative receptor with the surfactant protein (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 morphogenesis (29). Likewise, expression of a soluble FGFR dominant negative mutant blocked limb and lung development in vivo, findings identical to those in the FGF-10-null mice, supporting the primary role of FGFR signaling and FGF-10 in lung morphogenesis (6). Whereas FGFR3- and FGFR4-null mice did not have abnormalities in lung formation, double-null FGFR3/4 mice developed emphysema in the postnatal period, demonstrating a more subtle effect of FGFR3 and FGFR4 on postnatal lung growth (42). The ligands mediating FGFR3 and FGFR4 signaling have not been clarified. It therefore remains unclear how the precise temporal, spatial, and stoichiometric expression of FGF polypeptides mediate normal branching morphogenesis of the lung in vivo.

In the present study, conditional expression of FGF-10 was achieved utilizing the reverse tetracycline transactivator (rtTA) (11) that is expressed in respiratory epithelial cells under control of either the SP-C or Clara cell secretory protein (CCSP) promoters. Expression of FGF-10 in the fetal lung altered lung morphogenesis and caused focal pulmonary adenomas when induced in the postnatal period.

**EXPERIMENTAL PROCEDURES**

**Transgenic mice.** Permanent transgenic mouse lines bearing the SP-C-rtTA and CCSP-rtTA transgenes were established on an FVB/N background after oocyte injection of the plasmid constructs. The constructs consist of 3.7 kb of the human SP-C promoter (10) or 2.3 kb of the rat CCSP promoter (36) placed 5′ of the rtTA gene construct. The mouse FGF-10 cDNA (39) was inserted between the (teto)2-CMV promoter and the 3′-untranslated region of the bovine growth hormone gene (Fig. 1). The rtTA and (teto)2 constructs were kindly provided by Dr. Herman Bujard (Heidelberg) (11). Offspring of all founders were screened by Southern blot analysis; mice transmitting the “activator” rtTA transgene were bred to establish permanent CCSP-rtTA and SP-C-rtTA mouse colonies (40). Transgenic CCSP and SP-C-rtTA activator lines have been stable for more than a year in the vivarium. The target mice, (teto)2-CMV-FGF-10, were generated by oocyte injection of the plasmid DNA into the FVB/N strain, and founders were screened by PCR analysis. Heterozygous (teto)2-CMV-FGF-10 mice were viable and without observable abnormalities. Two separate target lines bearing the (teto)2-CMV-FGF-10 transgene (lines A and B) were chosen for breeding to the CCSP-rtTA and SP-C-rtTA activator mice, transmitting the genes with typical Mendelian inheritance patterns. All mice were maintained in a pathogen-free vivarium. Doxycycline (0.5 mg/ml) was administered in drinking water for the described time periods, the solution being changed three times per week.

**RT-PCR.** Tissues were homogenized in TRIzol reagent (Life Technologies), and RNA was isolated according to the manufacturer’s specification. RNA was treated with DNase before cDNA synthesis. RNA (5 μg) was reverse transcribed and then analyzed by PCR for total FGF-10, exogenous FGF-10, and β-actin mRNA. Transgene-specific primers for the mouse activating protein (VP) and FGF-10 transgene were designed to the (teto)2-CMV-FGF-10 transcript primer A in the CMV minimal promoter (5′ to 3′) GAC GCC ATC CAC GCT GTT; primer B in the FGF-10 cDNA (5′ to 3′) ATT TGC CTG CCA TTG TGC CAG, used for amplification, and compared with those amplified for β-actin. Total FGF-10 was measured using primers designed to amplify within the FGF-10 coding sequence.

![Design of transgenes for conditional expression of mouse (m) fibroblast growth factor (FGF-10)](http://ajplung.physiology.org/)
Histology, immunohistochemistry, and electron microscopy. To obtain fetal lung tissue, the fetuses were removed by hysterotomy after lethal injection of pentobarbital sodium 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 cmH$_2$O pressure via a tracheal cannula with the same fixative. Tissue was fixed overnight, washed in PBS, dehydrated through a series of alcohols, and embedded in paraffin. Tissue sections were stained for SP-B, proSP-B, thyroid transcription factor (TTF)-1, proSP-C, CCSP, and 5-bromo-2’-deoxyuridine (BrdU) as previously described (10). For electron microscopy, tissue was postfixed in 1% osmium tetroxide and evaluated as previously described (18).

In situ hybridization. Expression of FGF-10 mRNA was assessed by in situ hybridization using $^{35}$S-labeled riboprobes as previously described for fetal and adult lungs (40), the latter after inflation fixation at 25 cmH$_2$O pressure. Sense and antisense FGF-10 RNA probes were generated in pGEM32. Tissue was hybridized overnight at 50°C. Slides were coated with Kodak NTB-2 emulsion, exposed for 3–7 days, and developed with Kodak D19.

RESULTS

Generation of SP-C-rtTA, CCSP-rtTA, and (teto)$_7$CMV-FGF-10 transgenic mice. Transgenic CCSP-rtTA, SP-C-rtTA, and (teto)$_7$CMV-FGF-10 mice (heterozygous for each transgene) were viable and were produced in ratios predicted by Mendelian inheritance. Characteristics of the rtTA activator mice were recently described (40). In situ hybridization analysis of the lungs from SP-C-rtTA activator mice demonstrated that rtTA mRNA was selectively expressed in peripheral respiratory epithelial cells in the lungs of fetal [postconception (pc) day 15] and adult mice. In CCSP-
rtTA mice, rtTA mRNA was detected in both tracheobronchial and type II cells (40). Two independent lines of (teto)-CMV-FGF-10 target mice (lines A and B) were generated.

**Conditional expression of FGF-10 mRNA.** Transgene-specific FGF-10 mRNA was assessed by RT-PCR in lungs of young adult mice with and without addition of 0.5 mg/ml doxycycline in the drinking water. In adult double-transgenic CCSP-rtTA × (teto)-CMV-FGF-10 mice (“target” lines A and B), FGF-10 mRNA was undetectable in the absence of doxycycline but was markedly induced after oral doxycycline and reversed by withdrawal from doxycycline (Fig. 2). In CCSP-rtTA × (teto)-CMV-FGF-10 mice (line B), FGF-10 mRNA was detected in fetal and adult lung but transgene mRNA was detected only in postnatal lung from mice generated from line A. FGF-10 mRNA was detected in SP-C-rtTA × (teto)-CMV-FGF-10 (line A) adult double-transgenic mice in the presence or absence of doxycycline, the abundance of FGF-10 mRNA being increased by exposure to doxycycline; however, FGF-10 mRNA was not detected in fetal lung (pc day 18) with or without doxycycline in SP-C-rtTA × (teto)-CMV-FGF-10 line A offspring (data not shown). In contrast, double-transgenic mice generated by crossing SP-C-rtTA and (teto)-CMV-FGF-10 (line B) generally died at birth whether or not they were treated with doxycycline, and abundant FGF-10 mRNA was detected in lungs of these fetal mice at day 18 of gestation. Transgenic FGF-10 mRNA was not detected in other major organs, including liver, kidney, brain, testes, muscle, and heart, typical of the specificity of the CCSP and SP-C promoter elements, which are generally active only in respiratory epithelial cells in the lung (10, 36) (data not shown).

**Effects of FGF-10 on the fetal lung.** Histology of lungs from double-transgenic SP-C or CCSP-rtTA × (teto)-CMV-FGF-10 offspring (line B) was assessed in fetal mice treated with doxycycline (Fig. 3). At pc day 17, histological abnormalities observed in the FGF-10-expressing mice were similar with both SP-C- and CCSP-driven transgenes, consisting of marked adenomatoid hyperplasia with filling of peripheral and small conducting airways with hyperplastic epithelial cells. Whereas similar abnormalities were observed in all FGF-10-expressing fetal mice, variability in the sites and extent of hyperplasia was observed even in the same litter (Fig. 3), suggesting that the timing, extent, or levels of transgene expression may vary and influence phenotype. In general, the fetal lungs were highly cellular, consisting of dense lung parenchyma with characteristics of focal or diffuse pulmonary adenomas. Occasionally, cystic changes were observed in the lung parenchyma at pc day 17. Generally, epithelial cells lining the respiratory tubules of FGF-10-expressing mice stained intensely for proSP-C, TTF-1, and SP-B, consistent with the characteristics of type II epithelial cells (data not shown). Multiple adenomatous polyps were occasionally observed in the conducting airways of double-transgenic CCSP-rtTA pups on doxycycline at pc day 17 (Fig. 3B), and some of these cells stained for CCSP (data not shown). In contrast to CCSP-rtTA × (teto)-CMV-FGF-10 (line B) fetal pups, CCSP-rtTA × (teto)-CMV-FGF-10 (line A) pups had normal lung histology following doxycycline treatment; however, in situ hybridization analysis revealed a lack of mRNA expression in this line in the fetal, but not in the adult, mouse lung.

In situ hybridization demonstrated widespread expression of FGF-10 mRNA in respiratory epithelial cell in conducting airway and alveolar regions in fetal SP-C and CCSP-rtTA × (teto)-CMV-FGF-10 exposed to doxycycline (Fig. 4). FGF-10 mRNA was detected in fetal lung from CCSP-rtTA × (teto)-CMV-FGF-10 (line B) only in the presence of doxycycline but was detected in SP-C-rtTA × (teto)-CMV-FGF-10 (line B) in the presence of (teto)-CMV-FGF-10 target mice (lines A and B). Original magnification, ×40.
and absence of doxycycline. Endogenous mouse FGF-10 mRNA was not detected in control lung tissue under these same hybridization and autoradiographic exposures, but mouse FGF-10 mRNA was detected by RT-PCR in control lung (Fig. 4). Histological abnormalities and FGF-10 mRNA levels in SP-C-rtTA (teto)7CMV-FGF-10 (line B) lung were increased by administration of doxycycline to the dam (data not shown). SP-C-rtTA (teto)7CMV-FGF-10 (line B) mice generally did not survive postnatally, likely because of severe pulmonary malformations. In contrast, FGF-10 mRNA was not detected in the fetal lungs (pc day 17) of double-transgenic CCSP-rtTA (teto)7CMV-FGF-10 (line A), explaining the normal lung histology seen in these animals (data not shown). In general, histological abnormalities seen in the double-transgenic mice correlated with the level of expression of the transgene.

FGF-10 caused multifocal pulmonary tumors postnatally. In the absence of doxycycline, lung size and histology of double-transgenic mice from CCSP-rtTA (teto)7CMV-FGF-10 (lines A and B) were not different from the nontransgenic controls. In contrast, when weaning double-transgenic mice from either line A or B were treated with doxycycline in the drinking water for 2–4 wk, all double-transgenic mice developed multifocal, adenomatous lung tumors (Fig. 5). FGF-10 mRNA was readily detected in the tumors by in situ hybridization (Fig. 6). Abnormalities were confined to the lung, and the number and size of tumors were generally greater in double-transgenic mice from line B, consistent with greater signal intensity of the transgenic mRNA in line B (data not shown). Tumors were readily visible by gross inspection, and histological classification was consistent with multifocal lepidic and papillary tumors (Figs. 5–7). Tumor formation was extensive and observed in all double-transgenic mice treated with doxycycline but was never seen in wild-type, single-transgenic mice or double-transgenic mice from line B, consistent with greater signal intensity of the transgenic mRNA in line B (data not shown). Tumors largely resolved after removal of doxycycline, demonstrating the reversibility of the tumors induced by FGF-10. Figures are representative of 3 separate experiments with each line.
tration (Fig. 5) and often caused respiratory distress. Increased numbers of mononuclear cells were consistently observed in the lung parenchyma surrounding the tumors in all FGF-10-expressing mice. SP-C-rtTA (teto)7CMV-FGF-10 (line B) mice rarely survived postnatally, consistent with both the leaky expression of FGF-10 in SP-C-activated mice and the increased severity of lung malformations in (teto)7CMV-FGF-10 (line B) compared with line A.

Respiratory epithelial markers. Whereas much of the lung parenchyma was relatively unaffected, focal adenomas were readily observed throughout the lungs of mice expressing FGF-10 in the postnatal period. Epithelial cells in tumors from adult double-transgenic CCSP- and SP-C-rtTA-activated mice stained intensely for TTF-1, proSP-C, and proSP-B (Fig. 7). Generally, epithelial cells in the relatively normal lung parenchyma surrounding the lesions stained less intensely for each of these markers, but the intensity of staining for TTF-1, proSP-B, and proSP-C was increased in type II cells in the FGF-10-expressing mice compared with that in wild-type mice. Tumor cells were stained by both proSP-B and mature SP-B antisera (latter not shown), indicating that the cells processed proSP-B to the active SP-B peptide, a function restricted to type II epithelial cells in the normal lung (20). Consistent with this observation, the intensity of staining for proSP-C, a type II cell-specific marker, was increased in the tumors from both CCSP- and SP-C-driven double-transgenic mice while staining for endogenous CCSP, a marker of nonciliated bronchiolar cells, was not generally observed in the tumors from postnatal animals. BrdU labeling was selectively increased in the tumors from the double-transgenic mice (Fig. 8), but focal increased BrdU uptake was occasionally observed in less involved regions of the lung parenchyma.

Ultrastructure of FGF-10-induced tumors. Ultrastructural features of the FGF-10-induced tumors were typical of type II epithelial cell tumors (Fig. 9). Tumor cells were predominantly cuboidal and contained numerous lamellar bodies and extensive microvilli. Increased numbers of type II epithelial cells were often located along alveolar septa in less affected regions of the lung parenchyma, a finding generally not observed in normal lung.

Reversal of doxycycline-induced tumor formation. To assess the reversibility of the tumor formation, double CCSP-rtTA × (teto)7CMV-FGF-10 transgenic mice were placed on doxycycline for several weeks and withdrawn from doxycycline for 1–4 wk (line A) or on doxycycline for 12 days and withdrawn from doxycycline for 4 wk (line B).
cline for 6 days (line B). Dramatic tumor regression was observed in all animals after removal from doxycycline (Figs. 5 and 10) in association with the loss of FGF-10 mRNA (Fig. 2). Residual focal abnormalities with increased numbers of mononuclear cells were observed in the lung parenchyma, consistent with remodeling at the site of tumor regression (Fig. 10), and fibrotic changes were not observed after recovery.

Withdrawal from doxycycline caused rapid loss of cuboidal cell morphology, decrease in intensity, and extent of expression of TTF-1, proSP-C, and SP-B, consistent with loss of type II epithelial cell characteristics (Fig. 11). After removal from doxycycline, deoxyribonucleotidyltransferase dUTP nick end labeling (TUNEL) assay and electron microscopy of the tumors did not show evidence of large-scale apoptosis that could account for the rapid resolution of the tumors (data not shown).

DISCUSSION

FGF-10 mRNA was selectively and conditionally expressed in respiratory epithelial cells of the lungs of fetal and postnatal mice under control of doxycycline. Pulmonary morphogenesis was markedly perturbed by expression of FGF-10 in the fetal lung. In the postnatal lung, induction of FGF-10 mRNA caused extensive multifocal, papillary, and lepidic pulmonary adenomas lined by type II epithelial cells. Tumors completely regressed in association with a rapid decrease in the expression of type II cell markers after withdrawal of doxycycline. The effects of FGF-10 were similar to, but distinct from, those of FGF-7 in both fetal and postnatal lungs.

Effects of FGF-10 in lung morphogenesis. In the present study, ectopic expression of FGF-10 in the respiratory epithelium of fetal lung markedly perturbed lung morphogenesis and caused dense, adenomatous malformations. The abnormalities were similar to, but distinct from, those induced by FGF-7 (35, 40), the latter causing generalized hyperplasia in the adult and marked cystic changes in the fetal lung. Ultrastructural features of respiratory epithelial cells in the tumors from FGF-10 transgenic mice were characteristic of type II epithelial cells and stained in-
tensely for the differentiation markers TTF-1, proSP-C, and SP-B, consistent with a role for FGF-10 in respiratory epithelial differentiation.

Targeted disruption of the mouse FGF-10 gene produced mice lacking both limbs and lungs (25, 34). Taken together with the observation that FGF-10 mRNA is produced by the lung mesenchyme at restricted sites near branch sites of lung buds, FGF-10 signaling was hypothesized to play a critical role in branching morphogenesis of the lung. Considered in the light of failed lung formation in the FGF-10 gene-targeted mouse, the effects of ectopic expression of FGF-10 observed in the present study support the concept that precise temporospatial control of FGF-10 expression is required for normal branching morphogenesis.

Similar but distinct postnatal effects of FGF-10 and FGF-7. Findings in the FGF-10 double-transgenic mice were distinct from those in postnatal rodents treated with exogenous FGF-7 (41) or in transgenic mice in which FGF-7 was expressed in the respiratory epithelium throughout lung development (35). In contrast to the present findings in the FGF-10-expressing mice, organized tumor formation was generally not seen in mice expressing FGF-7 in the postnatal period (40). FGF-7 induced widespread respiratory epithelial cell hyperplasia but did not cause well-organized tumors. The finding that increased and ectopic expression of FGF-10 mRNA in fetal lung produced dense, adenomatous changes with mild cyst formation contrasted with the observations that FGF-7 caused massive pulmonary cysts that were lethal to the fetus by 15–16 days of gestation (35, 44). These findings may be related to differences in the extent and level of expression of the FGFs or to distinct effects of the different polypeptides on pulmonary cells; they may also indicate greater effects of FGF-7 on fluid and electrolyte transport in the fetal lung. Increased staining for TTF-1 and surfactant proteins and infiltration by alveolar macrophages were observed in both FGF-7- and FGF-10-expressing mice. Whether the latter finding represents a direct effect of the FGF family members on macrophage migration and proliferation or represents a response to increased cell turnover or tumor formation is unclear at present. The observation that both FGF-7 and FGF-10 enhanced TTF-1 and surfactant protein expression supports the concept that these polypeptides share signaling pathways in respiratory epithelial cells.

The mechanisms underlying the observed differences in the effects of FGF-7 and FGF-10 on pattern organization of lung tissues remain to be clarified. Effects of FGF-10 on the fetal lung may be limited by as yet unknown regulatory molecule(s) that limit synthesis, secretion, access, or signaling by FGF-10 in a manner distinct from FGF-7. Because the FGF-7 and FGF-10 transgenes are expressed in epithelial cells and not in mesenchymal cells as in wild-type mice, bioavailability of the two ligands or accessibility of the ligands to FGFR may be distinct in the transgenic mice. Interaction of FGF-7 and FGF-10 with heparan sulfate proteoglycans in the extracellular matrix or on the cell surface is distinct, effects of FGF-10 being enhanced by heparin, whereas those of FGF-7 are inhibited (15), although the inhibitory effects of heparin on the actions of FGF-7 are controversial (4). Likewise, whereas FGF-7 and FGF-10 share binding to the FGFR2-IIIb isoform, FGF-10 also binds to the FGFR1-IIIb isoform (21).

FGF-10 is sufficient to cause multifocal pulmonary tumors in the postnatal lung. Expression of FGF-10 caused multifocal, highly differentiated adenomas in vivo, demonstrating that doxycycline-induced FGF-10 was sufficient for production of pulmonary tumors. BrdU labeling was increased in FGF-10-induced tumors, likely indicating its stimulatory effect on cell proliferation. In the present study, FGF-10-induced tumors were detected within 1–4 wk after treatment with doxycycline. In line B, the tumors progressed rapidly, causing massive tumor infiltration and respi-
ratory distress. The extent of tumor formation correlated in general with the sites and levels of transgene expression. Despite multifocal tumors, much of the pulmonary parenchyma remained unperturbed by expression of FGF-10 in line A mice. This finding may be related to relatively more restricted sites of expression of the transgene. Tumors from all FGF-10-expressing mice consisted of highly differentiated pulmonary adenomas with immunohistochemical and ultrastructural features of type II epithelial cells. Staining for TTF-1, a homeodomain-containing transcription factor critical to lung morphogenesis and gene expression (5, 16), was increased in the tumors, and withdrawal from doxycycline was associated with a rapid change in cell morphology and immunostaining for TTF-1, proSP-C, and SP-B, all markers of type II cell differentiation. Likewise, type II epithelial cell features were noted in the hyperplastic epithelial cells in all of the tumors, whether generated by the SP-C or CCSP promoters. The paucity of CCSP staining in adult tumors suggests that FGF-10, even when expressed with the CCSP promoter, may selectively influence respiratory epithelial cell commitment or differentiation to type II epithelial cell subtypes. The characteristics of immunostaining of surfactant proteins and TTF-1 observed in FGF-10-expressing mice were similar to those induced by FGF-7 (35, 40).

Whether increased FGF signaling influences pulmonary tumorigenesis to cause clinical disease is unclear. Altered FGFR and FGF production have been associated with oncogenesis in a variety of organ systems. Non-small cell lung carcinomas, many with histological features similar to those presently observed in the FGF-10-expressing mice, represent an increasing subset of human lung cancers. Non-small cell tumors express FGFR, including FGFR1, FGFR2, FGFR3, and varying levels of FGF polypeptides, supporting their potential role in oncogenesis (3). In the present work, the tumors produced in the FGF-10 double-transgenic mice regressed following withdrawal of doxycycline; thus FGF-10 does not appear to be oncogenic during the time period studied. Regression was associated with rapid loss of cuboidal shape and markedly decreased expression of TTF-1, SP-B, and proSP-C, consistent with a change in cell differentiation caused by decreased FGF-10. TUNEL staining and electron microscopy did not support changes consistent with apoptosis as a primary cause of tumor regression after removal from doxycycline. Epithelial cells detached from the basal lamina of the tumors after removal of doxycycline, a process that may play a role in the resolution of the tumors.

Utility of the rtTA system for conditional expression in the lung in vivo. We recently generated permanent activator mouse lines that express rtTA under con-
control of the SP-C (expressed in distal bronchiolar and alveolar type II cells) and CCSP (expressed in non-ciliated columnar and alveolar respiratory epithelial cells) promoters (40). In preliminary studies from this laboratory, induction of transgene expression by doxycycline treatment of the dam was observed as early as pc day 14 with the CCSP promoter and as early as pc day 12.5 with the SP-C promoter (Perl, Tichelaar, and Whitsett, unpublished observations). These observations are consistent with previous studies demonstrating the expression of CCSP- and SP-C-driven transgenes in the developing lung (10, 43). The finding that rtTA and FGF-10 transgenic mRNAs were consistently detected in both conducting airways and alveolar type II cells in CCSP-rtTA offspring was somewhat surprising and distinct from the pattern of expression of other CCSP transgenes that generally target only the conducting airways, an observation likely related to positional effects modifying the sites of expression of CCSP-rtTA in this mouse line. Differences in the levels and extent of transgene expression due to positional effects may also influence the phenotype in mouse lines generated for this study. The failure to detect FGF-10 mRNA in the absence of doxycycline in CCSP-rtTA × (teto)7CMV-FGF-10 activator mice demonstrates the relatively tight regulation and cell specificity of this gene expression system in vivo. Both SP-C-rtTA and CCSP-rtTA activator mice induce target genes in a lung epithelial cell-restricted pattern under control of doxycycline and should be useful for the study of pulmonary development and function.

In conclusion, the present findings support the concept that increased expression of a single gene, FGF-10, was sufficient to induce organized tumors with type II epithelial cell characteristics in the lung in vivo. The similar but also distinct effects of FGF-7 and FGF-10 on the prenatal and postnatal lung suggest that both shared a unique pathway mediating FGF signaling by these two closely related ligands. Differences between the effects of heparin and glypican-1 on the bioactivity of acidic fibroblast growth factor and the keratinocyte growth factor. J Biol Chem 274: 36132–36138, 1999.


FGF-10 CAUSES PULMONARY ADENOMAS IN VIVO


