Published in final edited form as:

Dev Dyn. 2011 June ; 240(6): 1512–1517. doi:10.1002/dvdy.22610.

Foxn4 Influences Alveologenesis during Lung Development

Shengguo Li and Mengqing Xiang*

Center for Advanced Biotechnology and Medicine and Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School, 679 Hoes Lane, Piscataway, New Jersey 08854, USA.

Abstract

The terminal stage of lung development begins with the formation of the terminal sacs followed by subdivision of these sacs by septa into numerous alveoli to maximize the gas-exchange surface. This process requires coordinated action of various intrinsic and extrinsic factors as well as interaction of epithelial and mesenchymal cells. We show here that during murine lung development the *Foxn4* transcription factor is expressed in proximal airways by a subpopulation of postmitotic epithelial cells which are distinct from basal and ciliated cells and of which only a small fraction are Clara cells. Targeted inactivation of *Foxn4* causes dilated alveoli, thinned alveolar walls and reduced septa in the distal lung but no overt gross alterations in proximal airways. The alveolar defects in mutants may result from decreased PDGFA signaling and reduced surfactant SFTPB expression. These data together suggest that *Foxn4* may have a non-cell-autonomous role critical for alveologenesis during lung development.

Keywords

Foxn4; winged-helix/forkhead; transcription factor; lung; alveologenesis; proximal airway

INTRODUCTION

Development of the mammalian respiratory system begins with the formation of the tracheal and lung primordial buds from the ventral foregut and their septation from the esophagus. The lung primordia subsequently undergo several stages of branching morphogenesis including canalicular, saccular, and alveolar stages to develop into a tree-like system of mature airways and alveoli (Hogan and Yingling, 1998; Perl and Whitsett, 1999; Warburton et al., 2000; Cardoso and Lu, 2006). The proximal airways (trachea, bronchi and bronchioles) and distal alveoli are lined with distinct types of epithelial cells. The proximal epithelium consists primarily of ciliated and secretory (Clara) cells with or without basal and goblet cells depending on axial regions while alveoli are populated with type I and type II cells. The complex branching morphogenesis and cell differentiation process require coordinated activities of a variety of signaling molecules (e.g. FGFs, Shh, BMPs, Wnts and PDGFA) and transcription factors including homeodomain, forkhead/winged helix, zinc-finger, and HMG box proteins (Hogan and Yingling, 1998; Perl and Whitsett, 1999; Warburton et al., 2000; Cardoso and Lu, 2006). For instance, *Foxj1* is required for differentiation of ciliated cells and *Trp63* for specification of basal cells, while *Sox2* plays an essential role in the maintenance and differentiation of Clara, ciliated and goblet cells (Chen et al., 1998; Brody et al., 2000; Daniely et al., 2004; You et al., 2004; Tompkins et al., 2009). Alveologenesis of the distal lung occurs postnatally in mice and critically depends on PDGFA signaling for myofibroblast ontogeny and spreading (Bostrom et al.,

*Corresponding author: Dr. Mengqing Xiang Center for Advanced Biotechnology and Medicine 679 Hoes Lane Piscataway, NJ 08854
Tel: 732-235-4491 Fax: 732-235-4466 xiang@cabm.rutgers.edu.

1996; Lindahl et al., 1997). Despite our current understanding of the molecular events governing lung development, however, the subpopulations of epithelial cells and their molecular signatures and developmental regulation still remain to be further characterized.

Foxn4 belongs to the family of winged-helix/forkhead transcription factors (Carlsson and Mahlapuu, 2002), and is transiently expressed in a subset of progenitors during retinogenesis and spinal neurogenesis. In the mouse, previous gene targeting and gain-of-function studies have demonstrated a crucial role for Foxn4 in the generation of amacrine and horizontal cells during retinal development (Li et al., 2004). Foxn4 is also expressed by a subset of progenitor cells in the p2 domain during spinal cord development (Li et al., 2005). Its targeted deletion causes a fate-switch of V2b interneurons to V2a neurons whereas its misexpression promotes the V2b fate (Li et al., 2005; Del Barrio et al., 2007). Foxn4 acts genetically upstream of the basic helix-loop-helix (bHLH) factors Mash1 and Scf, both of which are required for specifying the V2b fate (Li et al., 2005; Muroyama et al., 2005). In addition, Foxn4 activates the expression of Dll4, a ligand for Notch signaling involved in suppressing the V2a fate (Del Barrio et al., 2007). In the zebrafish, Foxn4 has been found to control atrioventricular canal development by activating *tbx2b* gene expression (Chi et al., 2008). Here we demonstrate the expression of Foxn4 in a subpopulation of postmitotic cells in the developing proximal airway and provide evidence that it may indirectly impact on alveologenesis during murine lung development.

RESULTS AND DISCUSSION

Expression of Foxn4 in A Subpopulation of Postmitotic Cells in the Developing Proximal Airway

We investigated the expression pattern of Foxn4 during development of the mouse respiratory system by immunolabeling as well as by monitoring β -gal (β -galactosidase) expressed from the knock-in *lacZ* reporter in *Foxn4^{lacZ/+}* mice (Li et al., 2004). At E14.5, an anti-Foxn4 antibody (Li et al., 2004) immunostained a small number of cells in the epithelia of the trachea and esophagus but not in bronchi, bronchioles or distal epithelia (Fig. 1A,B,J,K). From E15.5 to P8, the antibody labeled a small set of cells in all epithelia of the trachea, bronchi, bronchioles, and esophagus but never in saccules or alveoli (Fig. 1C-H,L,M). Thus, Foxn4 expression appears to be restricted to the proximal airway in the developing respiratory system. Consistent with this, X-gal staining revealed the presence of β -gal in a small population of cells in the *Foxn4^{lacZ/+}* epithelia of proximal airway and esophagus but not in the distal airway at E15.5 and E16.5 (Fig. 1N-P).

In epithelia of the proximal airway, Trp63 is expressed prenatally in progenitor cells but postnatally only in differentiated basal cells (Daniely et al., 2004). Double-immunofluorescence revealed a complete absence of colocalization between Foxn4 and Trp63 in epithelial cells of the proximal airway at E15.5 and P5 (Fig. 1F,G), indicating that Foxn4-expressing cells are not progenitors or basal cells. Similarly, there was no colocalization between Foxn4 and β -tubulin IV, a marker for ciliated cells (Fig. 1H). In contrast, we found that approximately 23% of Foxn4-positive cells were also immunoreactive for Scgb1a1, a marker for Clara cells (Fig. 1I). These results together suggest that Foxn4 is expressed in a subset of proximal epithelial cells that are distinct from basal, ciliated and progenitor cells but overlap with Clara cells.

Given the expression of Foxn4 mostly in dividing progenitor cells during retinal and spinal cord development (Gouge et al., 2001; Li et al., 2004; Li et al., 2005), we tested whether this is also the case in the developing respiratory system by pulse-labeling S-phase cells by BrdU and then performing double-immunostaining using anti-Foxn4 and anti-BrdU antibodies. At E14.5, E15.5 and E18.5, we observed no colocalization of Foxn4 and BrdU in epithelial

cells of proximal airways and esophagus (Fig. 1J-M), indicating the expression of *Foxn4* only in postmitotic cells. Therefore, during development of the respiratory system, unlike its expression in neural tissues, *Foxn4* is expressed only in a small subset of postmitotic epithelial cells, consistent with its lack of colocalization with *Trp63* at E15.5 (Fig. 1F).

Abnormal Alveologenesis in *Foxn4* Null Mutant Mice

To investigate the potential role of *Foxn4* in lung development, we asked whether the cells that would normally express *Foxn4* were generated in *Foxn4^{lacZ/lacZ}* mutants (Fig. 2). In heterozygotes, X-gal staining of wholemount respiratory systems showed β -gal activity in the trachea, bronchi and bronchioles at E15.5 and P0 (Fig. 2A,C). A similar tree-like pattern of X-gal labeling was visible in *Foxn4^{lacZ/lacZ}* mutants (Fig. 2B,D). In cross sections of the mutant lung at E16.5, there was a subset of β -gal⁺ cells distributed along the epithelia of bronchi and bronchioles, just as those in the heterozygous lung (Fig. 2E-H), suggesting that the subpopulation of cells that would normally express *Foxn4* are produced in the proximal airway of *Foxn4* null mice. In P4-P8 control and mutant proximal airways, by immunofluorescence, there appeared to be a similar number of *Trp63*⁺ basal cells, β -tubulin IV⁺ ciliated cells, *Scgb1a1*⁺ Clara cells, and *CFTR*⁺ cells which are predominantly Clara cells (Engelhardt et al., 1994) (Fig. 3C-J). Both control and mutant bronchi contained approximately the same number of *Trp63*⁺ epithelial progenitors at embryonic stages (Fig. 3A,B). Scanning electron microscopy revealed the presence of similar ciliated and nonciliated cells in the epithelia of wild-type and mutant tracheas (Fig. 3K,L). Therefore, the proximal airways of *Foxn4* null mice appear to be grossly normal.

We next examined the morphology of distal airways by HE (hematoxylin and eosin) and fast red staining of lung sections from postnatal wild-type and mutant animals. Starting from P1, the distal air sacs in *Foxn4^{lacZ/lacZ}* mutants were visibly enlarged compared to those in the wild-type; in addition, the mutant lung exhibited saccular wall thinning and reduced septation (Fig. 4A,B). These saccular/alveolar defects worsened with increased developmental ages (Fig. 4C-H). At P8 in mutant lungs, the alveoli became up to several times dilated than those in the wild-type whereas alveolar walls became much thinner (Fig. 4E-H). Thus, unlike in the proximal airway, loss of *Foxn4* function results in overt defects in alveologenesis.

As a first step to determine the molecular basis underlying alveolar defects in the *Foxn4* mutant lung, we examined by immunostaining the expression levels of various proteins in postnatal distal airways. In mutant alveolar sacs, there is a significant downregulation of the expression of PDGFA (Fig. 5A,B), which is required for proper alveologenesis (Bostrom et al., 1996; Lindahl et al., 1997); whereas PDGFR α expression exhibits no obvious change (Fig. 5C,D). Similarly, there is a dramatic reduction in expression of the surfactant protein SFTPB in mutant alveoli although SFTPC expression barely decreases (Fig. 5E-H). In wild-type alveolar epithelia, α -smooth muscle actin (α -SMA) is particularly concentrated in septa (Fig. 5I); however, these densely labeled septa are largely missing from the mutant (Fig. 5J). The expression of elastin, on the other hand, does not seem to alter in the mutant (Fig. 5K,L). Thus, *Foxn4* inactivation differentially affects the expression of proteins involved in alveolar development.

The saccular/alveolar stage of lung development begins with the formation of the terminal sacs followed by subdivision of these sacs by septa into numerous alveoli to maximize the gas-exchange surface. The alveolar defects in *Foxn4* mutants indicate a crucial role for *Foxn4* during terminal lung development. Given the expression of *Foxn4* only in epithelia of the proximal airways, however, it appears that *Foxn4* is non-cell-autonomously involved in alveologenesis. This is in contrast with its cell-autonomous function in the retina and spinal cord, where it is transiently expressed in progenitor cells giving rise to sensory neurons that

it specifies (Li et al., 2004; Li et al., 2005; Del Barrio et al., 2007). During lung development, there are other nuclear regulatory factors that have both a cell-autonomous as well as non-cell-autonomous roles. For instance, despite the expression of *Hoxa5* only in mesenchymal cells and not in epithelial cells, its inactivation in mice results in mis-specification of numerous goblet cells in proximal epithelia and mis-positioning of myofibroblasts in postnatal lungs (Aubin et al., 1997; Mandeville et al., 2006).

It is unclear how *Foxn4* may indirectly impact on alveologenesis but there are at least two possibilities. First, *Foxn4* might control the expression or production of signaling molecules in a subset of proximal epithelial cells that in turn mediate its function in alveolar development. For instance, inactivation of *Pdgfa*, *Lfng*, *Notch*, and *Rarg/Rxra* genes all leads to alveolar defects similar to those present in *Foxn4* mutant lungs (Xu et al.; Bostrom et al., 1996; Lindahl et al., 1997; McGowan et al., 2000). It is therefore possible that *Foxn4* may be required for modulating the expression of genes involved in PDGFA, Notch and/or retinoid acid signaling. Consistent with this, we observed a significant decrease of PDGFA expression and associated dislocation of α -SMA⁺ myofibroblasts in *Foxn4* mutant alveoli (Fig. 5). It will be interesting to investigate whether Notch signaling is also altered in the mutant since *Foxn4* has been shown to activate *Dll4* expression in the spinal cord (Del Barrio et al., 2007). Besides signaling molecules, indirect regulation of SFTPB expression by *Foxn4* may additionally contribute to alveolar defects of the mutant because of the critical surface tension-reducing properties of surfactant proteins (Clark et al., 1995; Pison et al., 1996). Second, proper lung development including alveologenesis requires coordinated interaction between epithelial and mesenchymal cells and loss of *Foxn4* function might interfere with this critical interaction. Regardless of the mechanism, the alveolar defect may contribute to the severe postnatal lethality associated with *Foxn4* mutant mice (Li et al., 2004).

EXPERIMENTAL PROCEDURES

Immunostaining

Immunostaining of cryosections by fluorescence and color reaction was carried out as previously described (Xiang et al., 1993; Xiang, 1998; Li et al., 2004). In brief, dissected mouse tissues were fixed with 4% paraformaldehyde in PBS at 4°C for 30 min to 2 h depending on the size of tissue. The fixed sample was cryoprotected in 30% sucrose solution overnight and then embedded in OCT compound for cryosectioning. Antibodies used in this work are: anti-*Foxn4* (rabbit, 1:50) (Li et al., 2004); anti- β -tubulin IV (mouse, 1:50, Sigma); anti-Trp63 (p63) (mouse, 1:500, Santa Cruz); anti-Scgb1a1/CC-10 (goat, 1:1000, Santa Cruz); anti-BrdU (mouse, 1:50, BD Pharmingen); anti-CFTR (rabbit, 1:500, Alomone); anti- β -gal (rabbit, 1:5000, Cappel); anti-PDGFA (rabbit, 1:500, Santa Cruz); anti-PDGFR α (rabbit, 1:500, Santa Cruz); anti-SFTPB (sheep, 1:1250, Millipore); anti-SFTPC (rabbit, 1:500, Santa Cruz); anti-elastin (goat, 1:500, Santa Cruz); and anti- α -SMA (mouse, 1:500, Sigma).

BrdU Labeling

Staged pregnant mice were injected intraperitoneally with BrdU (5-bromo-2'-deoxy-uridine) solution at 75 μ g/g body weight. Two hours later the mice were sacrificed and tissues were dissected from collected embryos and processed for cryosectioning. Frozen sections were immunostained with anti-*Foxn4* prior to the immunofluorescent staining with anti-BrdU after 30 min treatment with 2 N HCl.

X-Gal and H&E Staining

For X-gal staining, fixed samples were incubated in PBS containing 0.02% Nonidet P-40 and 0.01% sodium deoxycholate for about 20 min. The staining was carried out in the same PBS solution containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 0.5 mg/ml X-gal at 37°C for a few hours to overnight. Stained sections were further counterstained with fast red according to the manufacturer's protocol (Vector Laboratories). For H&E staining, re-hydrated paraffin sections were stained in diluted Harris modified hematoxylin solution (Fisher Scientific) for 3-5 min, rinsed sequentially with water and saturated lithium carbonate solution, then followed by a one-minute incubation in Eosin/Phloxine solution. The stained section was rinsed with 70% ethanol and then gradually dehydrated and mounted for analysis.

Scanning Electron Microscopy

Scanning electron microscopy was carried out as described (O'Malley et al., 1995). In brief, dissected tracheas were fixed in 2% glutaraldehyde/PBS for 1 h at 4°C and then rinsed three times in PBS for 15 min each. The secondary fixation was carried out in 1% osmium tetroxide/PBS for another hour followed by rinse in ddH₂O for three times, 10 min each. After serial dehydration with 50%, 70%, 80%, 95% and 100% alcohol, the samples were processed for critical point drying and sputter coating. Finally, finished samples were analyzed with the Amray 1830I electron microscope.

Acknowledgments

We thank Kamana Misra, Kevin Jin, Haisong Jiang, and Min Zou for thoughtful comments on the manuscript. This work was supported by the National Institutes of Health (EY015777 and EY012020 to M.X.).

Grant sponsor: NIH; Grant number: EY015777 and EY012020

REFERENCES

- Aubin J, Lemieux M, Tremblay M, Berard J, Jeannotte L. Early postnatal lethality in Hoxa-5 mutant mice is attributable to respiratory tract defects. *Dev Biol.* 1997; 192:432–445. [PubMed: 9441679]
- Bostrom H, Willetts K, Pekny M, Leveen P, Lindahl P, Hedstrand H, Pekna M, Hellstrom M, Gebre-Medhin S, Schalling M, Nilsson M, Kurland S, Tornell J, Heath JK, Betsholtz C. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell.* 1996; 85:863–873. [PubMed: 8681381]
- Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD. Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. *Am J Respir Cell Mol Biol.* 2000; 23:45–51. [PubMed: 10873152]
- Cardoso WV, Lu J. Regulation of early lung morphogenesis: questions, facts and controversies. *Development.* 2006; 133:1611–1624. [PubMed: 16613830]
- Carlsson P, Mahlapuu M. Forkhead transcription factors: key players in development and metabolism. *Dev Biol.* 2002; 250:1–23. [PubMed: 12297093]
- Chen J, Knowles HJ, Hebert JL, Hackett BP. Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. *J Clin Invest.* 1998; 102:1077–1082. [PubMed: 9739041]
- Chi NC, Shaw RM, De Val S, Kang G, Jan LY, Black BL, Stainier DY. Foxn4 directly regulates tbx2b expression and atrioventricular canal formation. *Genes Dev.* 2008; 22:734–739. [PubMed: 18347092]
- Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BR, Weaver TE, Whitsett JA. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proc Natl Acad Sci U S A.* 1995; 92:7794–7798. [PubMed: 7644495]

- Daniely Y, Liao G, Dixon D, Linnoila RI, Lori A, Randell SH, Oren M, Jetten AM. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. *Am J Physiol Cell Physiol*. 2004; 287:C171–181. [PubMed: 15189821]
- Del Barrio MG, Taveira-Marques R, Muroyama Y, Yuk DI, Li S, Wines-Samuelson M, Shen J, Smith HK, Xiang M, Rowitch D, Richardson WD. A regulatory network involving Foxn4, Mash1 and delta-like 4/Notch1 generates V2a and V2b spinal interneurons from a common progenitor pool. *Development*. 2007; 134:3427–3436. [PubMed: 17728344]
- Engelhardt JF, Zepeda M, Cohn JA, Yankaskas JR, Wilson JM. Expression of the cystic fibrosis gene in adult human lung. *J Clin Invest*. 1994; 93:737–749. [PubMed: 7509347]
- Gouge A, Holt J, Hardy AP, Sowden JC, Smith HK. Foxn4—a new member of the forkhead gene family is expressed in the retina. *Mech Dev*. 2001; 107:203–206. [PubMed: 11520680]
- Hogan BL, Yingling JM. Epithelial/mesenchymal interactions and branching morphogenesis of the lung. *Curr Opin Genet Dev*. 1998; 8:481–486. [PubMed: 9729726]
- Li S, Misra K, Matisse MP, Xiang M. Foxn4 acts synergistically with Mash1 to specify subtype identity of V2 interneurons in the spinal cord. *Proc Natl Acad Sci U S A*. 2005; 102:10688–10693. [PubMed: 16020526]
- Li S, Mo Z, Yang X, Price SM, Shen MM, Xiang M. Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. *Neuron*. 2004; 43:795–807. [PubMed: 15363391]
- Lindahl P, Karlsson L, Hellstrom M, Gebre-Medhin S, Willetts K, Heath JK, Betsholtz C. Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. *Development*. 1997; 124:3943–3953. [PubMed: 9374392]
- Mandeville I, Aubin J, LeBlanc M, Lalancette-Hebert M, Janelle MF, Tremblay GM, Jeannotte L. Impact of the loss of Hoxa5 function on lung alveogenesis. *Am J Pathol*. 2006; 169:1312–1327. [PubMed: 17003488]
- McGowan S, Jackson SK, Jenkins-Moore M, Dai HH, Chambon P, Snyder JM. Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. *Am J Respir Cell Mol Biol*. 2000; 23:162–167. [PubMed: 10919981]
- Muroyama Y, Fujiwara Y, Orkin SH, Rowitch DH. Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. *Nature*. 2005; 438:360–363. [PubMed: 16292311]
- O'Malley BW Jr, Li D, Turner DS. Hearing loss and cochlear abnormalities in the congenital hypothyroid (hyt/hyt) mouse. *Hear Res*. 1995; 88:181–189. [PubMed: 8575993]
- Perl AK, Whitsett JA. Molecular mechanisms controlling lung morphogenesis. *Clin Genet*. 1999; 56:14–27. [PubMed: 10466413]
- Pison U, Herold R, Schurch S. The pulmonary surfactant system: Biological functions, components, physicochemical properties and alterations during lung disease. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 1996; 114:165–184.
- Tompkins DH, Besnard V, Lange AW, Wert SE, Keiser AR, Smith AN, Lang R, Whitsett JA. Sox2 is required for maintenance and differentiation of bronchiolar Clara, ciliated, and goblet cells. *PLoS One*. 2009; 4:e8248. [PubMed: 20011520]
- Warburton D, Schwarz M, Tefft D, Flores-Delgado G, Anderson KD, Cardoso WV. The molecular basis of lung morphogenesis. *Mech Dev*. 2000; 92:55–81. [PubMed: 10704888]
- Xiang M. Requirement for Brn-3b in early differentiation of postmitotic retinal ganglion cell precursors. *Dev Biol*. 1998; 197:155–169. [PubMed: 9630743]
- Xiang M, Zhou L, Peng YW, Eddy RL, Shows TB, Nathans J. Brn-3b: a POU domain gene expressed in a subset of retinal ganglion cells. *Neuron*. 1993; 11:689–701. [PubMed: 7691107]
- Xu K, Nieuwenhuis E, Cohen BL, Wang W, Canty AJ, Danska JS, Coultas L, Rossant J, Wu MY, Piscione TD, Nagy A, Gossler A, Hicks GG, Hui CC, Henkelman RM, Yu LX, Sled JG, Gridley T, Egan SE. Lunatic Fringe-mediated Notch signaling is required for lung alveogenesis. *Am J Physiol Lung Cell Mol Physiol*. 298:L45–56. [PubMed: 19897741]
- You Y, Huang T, Richer EJ, Schmidt JE, Zabner J, Borok Z, Brody SL. Role of f-box factor foxj1 in differentiation of ciliated airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2004; 286:L650–657. [PubMed: 12818891]

Fig. 1.

Expression of Foxn4 in a subset of proximal epithelial cells during lung development. **A-E:** Lung sections from the indicated developmental stages were immunolabeled with an anti-Foxn4 antibody. Only a small population of proximal epithelial cells were labeled by the antibody. **F-I:** Wild-type lung sections from the indicated stages were double-immunolabeled with the indicated antibodies. Arrows in (I) point to representative colocalized cells and the inset shows the corresponding outlined region at a higher magnification. Some Foxn4-positive cells co-express Scgb1a1. **J-M:** Esophageal (J) and lung (K-M) sections from embryos of the indicated stages pulse-labeled with BrdU were double-immunostained with anti-Foxn4 and anti-BrdU antibodies. There was no colocalization between Foxn4 and BrdU. **N-P:** β -gal activity was visualized by X-gal staining of E15.5 wholemount lung (N) and E16.5 lung sections (O,P) from *Foxn4^{lacZ/+}* mice. Note β -gal expression in the proximal airway tree (N) and subset of cells in epithelia of the bronchi, bronchioles and esophagus (O,P). Sections in (A-H,J-M) were counterstained with nuclear DAPI and those in (O,P) with fast red. A, alveolus; Bo, bronchiole; Bu, bronchus; D, distal epithelium; Es, esophagus; Tr, trachea. Scale bar = 12.5 μ m (G), 16.7 μ m (H), 25 μ m (A,B,D-F,I-M,P), and 50 μ m (C,O).

Fig. 2.

Grossly normal development of proximal airways in *Foxn4^{lacZ/lacZ}* mice. **A-H:** β-gal activity was visualized by X-gal staining of E15.5 and P0 wholemount lungs (A-D) and E16.5 lung sections (E-H) from *Foxn4^{lacZ/+}* and *Foxn4^{lacZ/lacZ}* mice. There is a similar tree-like pattern of X-gal labeling in both *Foxn4^{lacZ/+}* and *Foxn4^{lacZ/lacZ}* lungs (A-D); and a subset of β-gal⁺ cells are present in the epithelia of proximal airways in both control and mutant animals (E-H). Bo, bronchiole; Bu, bronchus; Tr, trachea. Scale bar = 25 μm (E-H).

Fig. 3.

Normal cell differentiation of proximal airways in *Foxn4^{lacZ/lacZ}* mice. **A-J:** Control and *Foxn4^{lacZ/lacZ}* lung sections from the indicated stages were immunolabeled with antibodies against Trp63 (A-D), β-tubulin IV (E,F), Scgb1a1 (G,H), and CFTR (I,J). There is similar immunoreactivity for Trp63, β-tubulin IV, Scgb1a1, and CFTR in the epithelia of control and mutant proximal airways. **K,L:** Scanning electron microscopy of P21 *Foxn4^{+/+}* and *Foxn4^{lacZ/lacZ}* tracheas showed the presence of both ciliated and non-ciliated epithelial cells in the wild-type and mutant. Sections in (A-J) were counterstained with nuclear DAPI. Bo, bronchiole; Bu, bronchus; Tr, trachea. Scale bar (in J) = 16.7 μm (E,F), and 25 μm (A-D,G-J). Scale bar (in L) = 10 μm (K,L).

Fig. 4. Abnormal alveolar development in *Foxn4^{lacZ/lacZ}* mice. **A-H:** Wild-type and mutant lung sections from the indicated postnatal stages were stained by hematoxylin-eosin (A-D,G,H) or fast red (E,F). Note the dilated alveoli, thinned alveolar walls and decreased septa in the mutant lung. Scale bar = 50 μ m (G,H), and 100 μ m (A-F).

Fig. 5. Altered protein expression in distal airways of *Foxn4*^{lacZ/lacZ} mice. **A-L:** wild-type and *Foxn4*^{lacZ/lacZ} lung sections from the indicated stages were immunostained with antibodies against PDGFA (A,B), PDGFR α (C,D), SFTPB (E,F), SFTPC (G,H), α -SMA (I,J), and elastin (K,L). There is reduced immunoreactivity for PDGFA, SFTPB and α -SMA in mutant alveoli. Arrows in (I) point to representative α -SMA-labeled septa. Sections in (A-H) were counterstained with nuclear DAPI. Bo, bronchiole; Bu, bronchus; Scale bar = 25 μ m (A-H), and 50 μ m (I-L).