β-Catenin Is Required for Specification of Proximal/Distal Cell Fate during Lung Morphogenesis*

Received for publication, June 4, 2003, and in revised form, July 24, 2003
Published, JBC Papers in Press, July 28, 2003, DOI 10.1074/jbc.M305892200

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The lungs are divided, both structurally and functionally, into two distinct components, the proximal airways, which conduct air, and the peripheral airways, which mediate gas exchange. The mechanisms that control the specification of these two structures during lung development are currently unknown. Here we show that β-catenin signaling is required for the formation of the distal, but not the proximal, airways. When the gene for β-catenin was conditionally excised in epithelial cells of the developing mouse lung prior to embryonic day 14.5, the proximal lung tubules grew and differentiated appropriately. The mice, however, died at birth because of respiratory failure. Analysis of the lungs by in situ hybridization and immunohistochemistry, using molecular markers of the epithelial and mesenchymal components of both proximal and peripheral airways, showed that the lungs were composed primarily of proximal airways. These observations establish, for the first time, both the sites and timing of specification of the proximal and peripheral airways in the developing lung, and that β-catenin is one of the essential components of this specification.

Lung morphogenesis depends upon precise regulation of reciprocal interactions between the endodermally derived respiratory epithelium and the surrounding lung mesenchyme. The primordial lung buds, derived from the foregut endoderm, invade the splanchnic mesenchyme at approximately embryonic day 9.5 to E9.5 in the developing mouse embryo. During the embryonic stage of development (E9.5 to E11.0), the buds undergo stereotypic branching to form the main stem and lobar bronchi. Extensive branching and budding of the airways continues throughout the pseudoglandular stage (E11.5 to E16.5), during which the intrapulmonary conducting airways and peripheral lung are formed. With advancing gestation, cytodifferentiation of distinct respiratory epithelial cell types occurs, producing the various cells lining the conducting (basal, ciliated, non-ciliated columnar, and neuroendocrine cells) and peripheral (alveolar Type I and Type II cells) airways. During the canalicular and saccular stages of lung development (E16.5 to E17.5 and E17.5 to postnatal day 4, respectively), the acinar tubules dilate into terminal alveolar sacs and the mesenchyme thins in association with formation of an extensive capillary network, forming the gas exchange region required for respiration after birth (1). Thus, formation of the lung is dependent upon precise temporal and spatial control of cell proliferation, migration, and differentiation, processes that are mediated by complex reciprocal interactions between cell types. Numerous signaling and transcriptional pathways, including those associated with fibroblast growth factors (FGFs), sonic hedgehog (Shh), bone morphogenetic protein 4 (Bmp4), vascular endothelial growth factors (Vegfs), thyroid transcription factor 1 (Titf1), and Wnts have been implicated in these interactions during lung morphogenesis (2–10).

The β-catenin gene encodes a 781-amino acid protein that regulates developmental processes mediating cell adhesion and gene expression (reviewed in Refs. 11 and 12). β-Catenin serves multiple roles in the maintenance of cell architecture, binding directly to the cytoplasmic tail of E-cadherin while simultaneously binding to α-catenin, a protein linked to actin filaments. β-Catenin also acts in complex intracellular signaling pathways that regulate gene transcription via members of the T cell factor/lymphoid enhancer factor (TCF/LEF) family of high mobility group domain-containing, DNA-binding proteins. The WNT/β-catenin signal transduction pathway controls a variety of biological processes, including embryonic patterning, development of the nervous system, and stem cell proliferation in Drosophila (reviewed in Ref. 13), as well as dorsal mesoderm induction and axis specification in Xenopus (14). In the mouse embryo, targeted mutagenesis of the β-catenin gene resulted in defects in anterior/posterior axis formation, as well as loss of mesoderm and head structures. Intercellular junctions were maintained in the β-catenin-targeted embryos by plakoglobin (also known as γ-catenin), which substituted for β-catenin in cell adhesion but not in transcriptional signaling (15).

The Wnt gene family encodes secreted glycoproteins that interact via seven transmembrane receptors of the Frizzled gene (Fzd) family. In the mouse, at least 19 Wnt genes and 10 Fzd genes have been identified (Wnt gene Homepage). WNT·
proteins signal through several potential signaling pathways (reviewed in Ref. 16). In the WNT/β-catenin pathway, specific WNT proteins interact with specific Fzd receptors, inhibiting glycogen synthase kinase 3β-dependent phosphorylation of β-catenin. Hypophosphorylated β-catenin accumulates in the cytoplasm, is translocated to the nucleus, and interacts with members of the TCF/LEF transcription factor family to become components of a transcription complex that regulates the expression of downstream target genes (reviewed in Refs. 13 and 15). Although a number of WNT ligands, Fzd receptors, and TCF/LEF proteins have been detected in lung tissue during embryonic development (17–21), the potential role of β-catenin in lung morphogenesis has not been determined. Because deletion of β-catenin is lethal before the initiation of lung development in the mouse embryo, a doxycline-induced, Cre recombinase (CRE)-mediated, homologous recombination strategy was utilized to specifically eliminate β-catenin expression in epithelial cells of the embryonic mouse lung.

EXPERIMENTAL PROCEDURES

Transgenic Mice—Floxed β-catenin mice were previously generated by insertingloxP sites into introns flanking exons 3 and 6 (22). Homologous recombination betweenloxP sites was accomplished using thetetO-CMV-Cre transgene (23). For long-saccouxcyline-induced recombination, the surfactant protein CCreversing tetracycline transactivator (SP-C-rtTA) or Clara cell secretory protein/reverse tetracycline transactivator (CCSP-rtTA) transgenes were used. In this system, continuous administration of doxycycline to the dam initiates recombinase of floxed alleles prior to lung bud formation, targeting most peripheral airways as well as subsets of tracheobronchial epithelial cells (24, 25). The 3.7-kilobase (kb) human SP-C promoter is expressed selectively in pulmonary epithelial cells in the embryonic lung and in alveolar and bronchial epithelial cells after birth (26). The 2.3-kb rat CCSP promoter is first expressed at E14.5–E15 in epithelial cells lining the trachea, bronchi, and bronchioles, as well as subsets of Type II cells later in development (27). Compound mutant animals (SP-C-rtTA/lox, tetO-CMV-Crelox/rtlox, β-cateninflx/lox or CCSPlox/rtlox or tetO-CMV-Crelox/rtlox, β-cateninflx/lox) were generated by breeding. Littermates of all other genotypes served as controls. Staging of embryos was based on the day of detection of a vaginal plug, which was then designated as embryonic day E0.5. Pregnant females were maintained on doxycycline-containing food (625 mg/kg; Harlan Teklad, Madison, WI) and water (0.5 mg/ml; Sigma) from E0.5 until the time of sacrifice. Litters were maintained on doxycycline-containing food after weaning. Mice were genotyped by PCR for the β-catenin alleles, as well as for the SP-C-rtTA and CCSP-rtTA transgenes, previously described (22, 28). CRE forward (5TGCAGCAGAATGAGCAGAATG-3’) and reverse (5T-AGAGACGGAATATCCATCGCTCG-3’) primers were used to genotype mice for the tetO-CMV-Cre transgene. PCR parameters used were: 30 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by a 7-min extension at 72°C. Mice used in this study were housed and maintained in pathogen-free conditions according to protocols approved by the Institutional Animal Care and Use Committee at Cincinnati Children’s Hospital Research Foundation. Pregnant dams were anesthetized with a mixture of ketamine, acepromazine, and xylazine, and then exsanguinated by severing the inferior vena cava and descending aorta. Fetuses were removed, the chest cavity opened, and the tissue fixed in 4% paraformaldehyde. The lungs of postnatal mice were inflation-fixed as previously described (29).

Immunohistochemistry—At least three to five compound mutant animals and littermate controls at E13.5, E14.5, E15.5, and E16.5 were analyzed for each immunohistochemical stain. Antibodies used were generated to: pro-SP-C (1:1000, rabbit polyclonal, AB13428, Chemicon), CCSP (1:7500, rabbit polyclonal, kindly provided by Dr. Barry Stripp, University of Pittsburgh), β-tubulin III (1:200, mouse monoclonal, M2178C, Biogenex), FOXJ1 (1:4000, rabbit polyclonal, kindly provided by Dr. Robert Costa, University of Illinois), cdc25C gene-product (1:4000, rabbit anti-rat polyclonal, Sigma), platelet endothelial cell adhesion molecule (PECAM) (1:500, rat polyclonal, clone CD31, BD Pharmigen), and α-smooth muscle actin (α-SMA) (1:20000, mouse monoclonal, clone 1A4, Sigma). Biotinylated secondary antibodies and a streptavidin-biotin-peroxidase detection system (Vector Laboratories, Inc.) were used to localize the antibody-antigen complexes in the tissues, as previously described (30). A mouse-on-mouse blocking kit (Vector Laboratories, Inc.) was used with primary mouse monoclonal anti-

FIG. 1. Doxycycline-inducible deletion of β-catenin in triple transgenic mice. Triple transgenic mice were generated that express the rTA protein specifically in epithelial cells of the lung, using theSP-C or CCSP promoter (latter not shown). Interacting with doxycyline, rTA activates expression of the (tetO)-CMV-Cre recombinase transgene. CRE recognizes theloxP sites present in the β-catenin locus, causing homologous recombination and deletion of genomic DNA containing exons 3–6 to generate a null mutant. Dams were treated with doxycycline from E0.5 until the day of killing.

bodies. Antigen detection was enhanced with nickel-diaminobenzidine and Tris-cobalt, followed by counterstaining with Nuclear Fast Red. For β-catenin immunostaining, a mouse monoclonal antibody (1:50, clone 14, BD Biosciences) was used. Unmasking was performed using an antigen unmasking solution (Vector Laboratories, Inc.). Primary antibody was incubated with sections overnight at room temperature, then washed three times with phosphate-buffered saline containing 0.1% Tween 20. Secondary antibody (1:200, goat anti-mouse horseradish peroxidase-conjugated) was added to the sections and incubated for 1 h at room temperature. After washing, slides were developed using a commercially available kit (Vector Laboratories, Inc.). No counterstaining was used. To detect mitotic cells, pregnant dams were injected intraperitoneally with BrdUrd labeling reagent, 100 μl per 10 g of body weight (Zymed Laboratories Inc.). Fetuses were harvested 2 h after injection. Paraffin-embedded tissues were stained for BrdUrd incorporation using the Zymed BrdUrd staining kit (Zymed Laboratories Inc.).

In situ Hybridization—In situ hybridization was performed using [35S]UTP or -CTP-labeled riboprobes for SP-A (a 850-bp mouse cDNA) (31), SP-B (32), SP-C (26), and Vegfa (33). Pod1 (a 594-bp mouse cDNA, IMAGE clone W08124), and β-catenin (a 324-bp mouse cDNA subcloned into pCRII, Invitrogen). In situ hybridizations were performed at E14.5, E15.5, and E16.5 as previously described (26). Lung whole mount in situ hybridization was performed at E13.5 as previously described (34), using digoxigenin-labeled riboprobes for Tgfβ (a 2.2-kb mouse cDNA from the Whittsett laboratory), Bmp4 (a 1.55-kb mouse cDNA, a gift from Dr. Brigid Hogan, Duke University), Shh (a 642-bp mouse cDNA, a gift from Dr. Andrew McMahon, Harvard University), and Fgfl0 (a 743-bp mouse cDNA, a gift from Dr. Nobuyuki Itoh, Kyoto University).

RESULTS

Compound Mutant Mice—CCSPlox/rtlox or rtlox, (tetO)-CMV-Crelox/rtlox or rtlox, β-cateninflx/lox, andSP-C-rtlox, (tetO)-CMV-Crelox/rtlox or rtlox, β-cateninflx/lox (termed compound mutant mice) were generated by breeding CCSPlox/rtlox, (tetO)-CMV-Crelox/rtlox or SP-Clox/rtlox, (tetO)-CMV-Crelox/rtlox mice to β-cateninflx/lox mice. The strategy used for the doxycycline-induced CRE/loxP system is outlined in Fig. 1. The reverse tetracycline transactivator gene (rtTA) was expressed using the 3.7-kb human SP-C promoter or the 2.3-kb rat CCSP promoter (latter not shown). In the presence of doxycycline, the rTA protein binds to the (tetO)-CMV promoter, thus activating the expression of the CRE protein, which recognizesloxP sites, causing recombination and deletion of genomic sequences including exons 3–6 of the β-catenin gene.

Deletion of β-Catenin Causes Lung Malformations—All SP-C compound mutant mice that had been maintained on doxycy-
null
extent and sites of deletion of β-catenin in the pulmonary epithelium was observed at all ages analyzed (E13.5, E14.5, E16.5, and E18.5) (data not shown). β-Catenin was not deleted in the pulmonary mesenchyme, consistent with the use of epithelial-specific promoters used to drive rtTA expression. These results were confirmed by in situ hybridization analysis for β-catenin expression (data not shown).

In the control litters at E18.5, CCSP immunostaining was detected in the bronchi and bronchioles of conducting airways, but not in the peripheral acinar tubules and buds (Fig. 4A). In the lungs of SP-C compound mutant animals, a dramatic increase in CCSP-positive cells was observed, consistent with the increased formation of bronchiolar tubules (Fig. 4B). Likewise, FOXJ1 and β-tubulin IV, both markers of ciliated cells that are normally restricted to the conducting airways (Fig. 4, C and E), were detected in the atypical bronchiolar tubules (Fig. 4, D and F). Pro-SP-C immunostaining was detected in alveolar Type II cells in alveolar sacculles of the control mice (G), but was markedly reduced or absent in the compound mutant mice (H). Scale bars: 500 μm in A and B; 100 μm in C, D, G, and H; and 50 μm in E and F.

In situ hybridization analysis for SP-A (A and B), SP-B (C and D), SP-C (E and F), and VegfA (G and H) mRNAs. Expression in control lungs at E18.5 are shown in A, C, E, and G. In mutant animals, expression of SP-A (B), SP-C (F), and VegfA (H) mRNAs was greatly reduced or absent, indicating the lack of peripheral epithelial cell differentiation. In contrast, SP-B mRNA, which is expressed in epithelial cells of both the conducting airways and the lung periphery at E18.5 in controls (C), was detected in the abnormal bronchiolar tubules in the β-catenin compound mutant mice (D), consistent with its expression in conducting airways at this time of development. Scale bar: 500 μm.
mice, SP-A, SP-C, and VegfA are normally expressed in alveolar Type II cells, whereas SP-B is expressed in epithelial cells of both the conducting airway and gas exchange region (Figs. 5, A, C, E, and G). In SP-C compound mutant mice, the sites of SP-A, SP-C, and VegfA expression were reduced (Fig. 5B, F', and H) and, in some cases, absent (data not shown). In contrast, SP-B mRNA was expressed in the bronchiolar and residual alveolar saccula, consistent with its expression in both conducting and peripheral airways of the normal lung (Fig. 5D).

Deletion of β-Catenin Disrupts Peripheral Vasculogenesis—While PECAM immunostaining was extensive in the peripheral regions of the normal lung at E18.5 (Fig. 6A), staining was markedly reduced in the SP-C compound mutant mice, indicating decreased peripheral vessel formation (Fig. 6B). Immunostaining for α-SMA, which is normally detected in myofibroblasts surrounding the conducting airways (Fig. 6C), was detected in the stroma surrounding the atypical bronchiolar tubules in the β-catenin compound mutant mice (Fig. 6D).

Tif1f, Bmp4, Shh, Fgf10, and Pod1 Expression Is Unaltered in Compound Mutant Lungs—Whole mount in situ hybridization analysis for Tif1f, Bmp4, Shh, and Fgf10 mRNAs was performed at E13.5. Tif1f is normally expressed in epithelial cells of the trachea and bronchiolar tubules at E13.5 (Fig. 7A) and required for formation of the peripheral lung (35). Although Tif1f expression was detected in the bronchiolar tubules of the SP-C compound mutant lung at E13.5, the number of tubules was reduced, indicating a defect in branching morphogenesis (Fig. 7B). The tips of the bronchiolar tubules were enlarged and poorly branched. Likewise, Bmp4 and Shh mRNAs were detected in distal lung epithelial cells of control and compound mutant embryos (Fig. 7, C–F). Fgf10, normally expressed in the mesenchyme near the tips of lung buds at E13.5 (Fig. 7G), was also still present at the tips of the bronchiolar tubules after deletion of β-catenin (Fig. 7H). Expression of Pod1, a basic-helix-loop-helix transcription factor, was detected in the pulmonary mesenchyme of both compound mutant and control animals at E14.5, E16.5, and E18.5 by in situ hybridization of lung sections (data not shown).

Cell Proliferation Is Unchanged but Nuclear Fragmentation Is Increased in Compound Mutant Lungs—Cell proliferation indices were estimated after BrdUrd injection of the dam prior to sacrifice at E14.5 and E18.5. BrdUrd labeling indices in the bronchiolar epithelium were unchanged in SP-C compound mutant mice in comparison to littermate controls (Fig. 8, A–D). Nuclear fragmentation was observed in the lung mesenchyme of compound mutant animals at E14.5, consistent with apoptosis. The number of fragmented nuclei in the lung mesenchyme was increased from 0.24 fragmented nuclei per lobe (n = 17) in control animals (Fig. 8E) to 14.5 fragmented nuclei per lobe (n = 12) in SP-C compound mutants (Fig. 8F). Nuclear fragmentation was not observed in epithelial cells in control or compound mutant mice. In addition, there was no evidence of necrosis or inflammation in the lungs of compound mutant mice at any gestational age examined.

**DISCUSSION**

Deletion of β-catenin in epithelial cells of the embryonic lung disrupted lung morphogenesis, restricting formation and differentiation of the peripheral lung and enhancing formation of the conducting airways. While the correct number of main stem bronchi and pulmonary lobes formed in the floxed β-catenin mutant mice, branching of secondary bronchi was altered and the number of small peripheral acinar tubules and terminal saccula was markedly reduced. The atypical bronchiolar tubules found in these mice were lined by pseudostatified columnar epithelium that expressed SP-B and stained positive for CCSP, β-tubulin IV, and FOXJ1, but lacked proSP-C immunostaining and VegfA mRNA expression. These bronchiolar tubules often extended to the pleural surfaces. Tubular diameter was increased and small terminal bronchiolae and acinar structures failed to form, resulting in a marked reduction of peripheral alveolar ducts and sacculae. The stroma surrounding the atypical bronchiolar tubules stained for α-smooth muscle actin, whereas PECAM staining (a marker of alveolar capillaries) was reduced, consistent with the proximalization of the mesenchyme surrounding the tubules. In the normal lung, nuclear staining for β-catenin was detected most frequently in epithelial cells of the peripheral lung structures at E13.5 to E16.5. In contrast, β-catenin was detected primarily in cell membranes in the more proximal conducting airway tubules during these stages of development. Deletion of β-catenin from E14.5 to E15.5 and thereafter, with the CCSP-rtTA compound mice, did not alter lung structure or postnatal survival. Taken together, β-catenin expression in the respiratory epithelium of the em-
bryonic lung is required for the growth and differentiation of peripheral epithelial cell progenitors.

The SP-C-rtTA conditional system utilized to delete β-catenin is active primarily in progenitor cells of the peripheral lung, but is not highly active in the trachea or main stem bronchi. The timing and extent of recombination with the SP-C-rtTA/tetO-Cre system has been previously documented, utilizing floxed lacZ alleles to activate alkaline phosphatase or green fluorescent protein (25). When maintained on doxycycline, recombination in this system was widespread, and often complete, occurring before the formation of proximal lung buds on E9.0. Recombination extended to subsets of bronchial and lower tracheal epithelial cells in a pattern established by E10.5 to E12.5. In contrast, expression of CCSP-rtTA in respiratory epithelial cells was first detected at E14.5–E15.5, which caused widespread recombination in the conducting airways when dams were continuously treated with doxycycline (Ref. 24, and data not shown). The sites and extent of deletion of β-catenin, as assessed by immunohistochemistry, were consistent with the activities of the SP-C and CCSP promoter elements used to express the rtTA protein. In the present studies, β-catenin was deleted in subsets of cells forming the peripheral lung early in lung morphogenesis. Nevertheless, because branching of the main stem bronchi and lobe formation was not perturbed in the SP-C compound mutant mice, β-catenin does not appear to be required for initial bronchial branching. Because branching morphogenesis and budding after E11.5 to E12.0 normally results in expansion of both conducting airways and peripheral acinar structures, deletion of β-catenin appeared to limit formation and/or differentiation of secondary/tertiary branching. These findings are distinct from those in which Titf1, Fgf10, and FgfR2IIb were deleted, each resulting in loss of pulmonary lobes and complete arrest of branching morphogenesis (36–39), and indicating the failure of commitment and/or survival of progenitor cells required for formation of the lung periphery. The continued outgrowth of the bronchiolar tubules after β-catenin deletion supports the concept that cell fate decisions, rather than survival, was dependent upon β-catenin expression. Likewise, the abnormal tubules are lined by epithelial cells lacking β-catenin, demonstrating that β-catenin is not required for their survival and proliferation.

Deletion of β-catenin caused a remarkable increase in conducting airways with large lumenal diameter, but did not completely abrogate proximal-distal patterning. Although the number of lung buds was decreased, expression of Titf1, Bmp4, and Shh was maintained in epithelial cells at the tips of the abnormal bronchiolar tubules. Complementary mesenchymal expression of Fgf10 at the tips of the lung buds was maintained but reduced in relationship to the decrease in numbers of lung buds. The relative intensity of hybridization signals for these mRNAs was also maintained at the tips of the lung buds. The structural changes in the lungs of the β-catenin mutant mice were distinct from those seen in Titf1, Shh, and Fgf10 null mice (6, 35, 37), wherein lobar branching was blocked. Deletion of Fgf10 (37), FgfR2IIb (36), expression of an FGF inhibitor (a mutant, soluble FGF receptor or FGF-RPc) or Sprouty (40, 41) inhibited both lobar branching and subsequent formation of peripheral lung structures, resulting in markedly hypoplastic lungs. Thus, in contrast to the present finding, elongated bronchiolar tubules with features of conducting airways were not observed after disruption of FGF, Shh, or TTF-1 pathways. Continued growth and proliferation of the abnormal bronchiolar tubules in β-catenin compound mutant mice supports the concept that β-catenin plays a critical role in the cell fate decision during programming of proximal versus peripheral respiratory epithelial cells, but is not required for primary lobar branching or continued growth of bronchiolar tubules.

Neither postnatal survival nor lung structure was perturbed when β-catenin was deleted using the CCSP promoter, an element that is highly active in conducting airways, trachea, bronchi, and bronchioles, from E14.0 to E15.0 and thereafter (24, 27), perhaps indicating the importance of β-catenin prior to but not after E14.0 to E15.0 for specification of peripheral lung.
structures. Alternatively, CCSP may have caused deletion of β-catenin in a subset of cells that are not critical for formation of peripheral airways and sacculae, although most respiratory epithelial cells in conducting airways are targeted in this system. In large airways at E14.0 to E18.5, β-catenin was primarily membrane associated and was not noted in the nuclei, consistent with previous findings (21). At the same time, nuclear β-catenin staining was more prominent in epithelial cells of the peripheral lung. The paucity of nuclear β-catenin staining in proximal airways from E13.5 and thereafter, and its relative abundance in peripheral acinar tubules and buds supports its role in commitment of peripheral lung progenitor cells. Nuclear β-catenin staining was most abundant in the lung periphery and decreased in the canicular period from E16.5 to E17.5. This pattern of expression is similar, but not identical, to that of TTF-1, FOXa2, BMP4, and SHH (4, 30, 35, 42), all known to play important roles in peripheral lung morphogenesis.

At least nine Wnt genes are expressed in the mesenchyme and/or epithelium of the lung. The biological function of a number of Wnt genes has been determined through the generation of null mutant mice. Wnt7b is normally expressed in epithelial cells of the lung periphery (43). Wnt7b null mutant mice died of respiratory failure at birth. Defects were observed in proliferation of lung mesenchyme resulting in lung hypoplasia. Severe defects were also observed in the smooth muscle compartment of major pulmonary vessels, resulting in rupture of major blood vessels (8). Wnt5a is normally expressed in both the mesenchymal and epithelial compartments of the developing lung. Wnt5a null mutant mice also die perinatally. Truncation of the trachea and overexpansion of peripheral airways and delayed lung maturation were observed in the Wnt5a null mice (5).

Many of the signaling components known in the WNT pathway are present in the embryonic lung, including a number of WNTs, β-catenin, Fzds, AXIN, glycogen synthase kinase 3β, TCFs, and LEF. Whereas targeted deletion of Wnt7b and Wnt5a altered lung morphogenesis, pulmonary findings in those mice were distinct from that presently observed, adding to the complexity of involvement of the WNT pathways in lung morphogenesis (5, 8). Whereas the mechanisms underlying the abnormalities in lung morphogenesis in the β-catenin deleted mice are unknown, severe lung abnormalities with loss of peripheral structures were observed after deletion of Pod1, a transcription factor expressed in the pulmonary mesenchyme (44). Alterations in expression of Pod1, however, were not observed in SPC compound mutant mice, suggesting that β-catenin does not indirectly regulate Pod1 expression in the mesenchyme. Likewise, overexpression of Gremlin, a BMP-4 inhibitor, the BMP antagonist Xnoggin, or a dominant negative BMP receptor (dnAlk6) in distal lung epithelium also caused alterations in proximal-distal patterning (45, 46).

Reciprocal signaling between epithelial and mesenchymal compartments of the lung is critical for branching morphogenesis and proximal-distal patterning of the lung. The present study demonstrates that β-catenin expression in the embryonic respiratory epithelium is required for commitment of progeni-


β-Catenin Signaling and Lung Morphogenesis

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