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Wnt/β-catenin signaling acts upstream of N-*myc*, BMP4, and FGF signaling to regulate proximal–distal patterning in the lung

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Abstract

Branching morphogenesis in the lung serves as a model for the complex patterning that is reiterated in multiple organs throughout development. β-catenin and Wnt signaling mediate critical functions in cell fate specification and differentiation, but specific functions during branching morphogenesis have remained unclear. Here, we show that Wnt/β-catenin signaling regulates proximal–distal differentiation of airway epithelium. Inhibition of Wnt/β-catenin signaling, either by expression of Dkk1 or by tissue-specific deletion of β-catenin, results in disruption of distal airway development and expansion of proximal airways. Wnt/β-catenin functions upstream of BMP4, FGF signaling, and N-*myc*. Moreover, we show that β-catenin and LEF/TCF activate the promoters of BMP4 and N-*myc*. Thus, Wnt/β-catenin signaling is a critical upstream regulator of proximal–distal patterning in the lung, in part, through regulation of N-*myc*, BMP4, and FGF signaling.

Keywords: Wnt; β-catenin; Dickkopf-1; BMP; FGF; Lung development

Introduction

Lung development in the mouse begins at approximately E9.5 when the trachea branches from the anterior foregut (for review, see (Warburton et al., 2000). Cellular differentiation along the proximal–distal axis of the lung is highly patterned and is regulated by several distinct signaling pathways, including members of the FGF and BMP pathways (Bellusci et al., 1997; Sekine et al., 1999; Weaver et al., 1999, 2000). Progenitor cells within distal airway epithelium generate type 1 and type 2 pneumocytes, which populate the alveolar airways and are required for the thin gas exchange interface in the airway lumen and surfactant protein production, respectively. Type 2 pneumocytes are thought to be progenitor cells within the adult lung alveolus, generating type 1 cells through a poorly understood process and also regenerating additional type 2 cells after lung injury (Borok et al., 1998; Danto et al., 1995; Qiao et al., 2003). Disruption of distal airway development and type 2–type 1 cell differentiation is associated with bronchopulmonary dysplasia (BDP), a common disorder that causes severe lung dysfunction (for a review, see (Demayo et al., 2002; Whitsett and Zhou, 1996).

Ligands and receptors for both FGF and BMP signaling are expressed at high levels in the distal airways, and several

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studies have demonstrated a critical role for these pathways in lung airway branching. Loss of either FGF10 or FGF receptor signaling results in severe abrogation of lung airway branching (Bellusci et al., 1997; Min et al., 1998; Peters et al., 1994; Sekine et al., 1999). This has been postulated to be due to the requirement of FGF signaling in distal epithelial cell proliferation and outgrowth (Bellusci et al., 1997; Clark et al., 2001). BMP signaling appears to play a different role. Transgenic mice expressing inhibitors of BMP signaling such as gremlin, noggin, or a dominant-negative BMP receptor Ib display a disruption of proximal–distal patterning in the lung where distal epithelial differentiation is inhibited while proximal differentiation is promoted (Lu et al., 2001; Weaver et al., 1999). BMP4 gain of function in the lung results in decreased epithelial proliferation and differentiation (Bellusci et al., 1996).

The Wnt pathway has also been implicated in regulating lung airway development. Wnts regulate developmental processes through several distinct pathways (for a review, see (Pandur et al., 2002). The best studied of these pathways, called the canonical pathway, involves the binding of Wnt ligands to a co-receptor complex consisting of frizzled and LRP5/6 proteins. This interaction inhibits the GSK-3β kinase, which results in a reduction in β-catenin phosphorylation, stabilizing this protein, leading to its accumulation in the nucleus. β-catenin binds to members of the LEF/TCF transcription factor family and activates down-stream targets of Wnt signaling. Through this pathway, Wnt signaling regulates several developmental processes including cell proliferation, migration, and differentiation.

Multiple components of the Wnt pathway are expressed in the lung, and recent reports have implicated several of these in regulating diverse aspects of lung morphogenesis. Inactivation of Wnt7b results in decreased airway branching leading to pulmonary hypoplasia as well as defects in lung vascular smooth muscle integrity (Shu et al., 2002). Wnt5a null mice exhibit defects in late lung maturation and air saccule development (Li et al., 2002). More recently, conditional inactivation of β-catenin in the airway epithelium of the lung has demonstrated a key role for this Wnt signaling component in differentiation of distal airway epithelium (Mucenski et al., 2003). However, since β-catenin has a dual role in both Wnt signaling and cell adhesion processes, the disruption in distal airway development in these mice cannot be specifically attributed to its role in Wnt signaling.

The precise role of Wnt/β-catenin signaling in the lung and how this pathway interacts with other signal transduction pathways such as BMP and FGF signaling is unclear in the lung, although earlier reports suggested that these pathways were not perturbed upon loss of Wnt/β-catenin activity (Mucenski et al., 2003). In this report, we show that canonical Wnt/β-catenin signaling is highly active in early lung airway development with the highest levels in distal airway epithelium. To precisely determine the role of Wnt/β-catenin signaling in lung morphogenesis, we expressed Dickkopf-1 (Dkk1), a specific inhibitor of canonical Wnt signaling and deleted β-catenin in the airways of the developing lung. We find that Dkk1 represses distal airway epithelial differentiation while at the same time causing an expansion of proximal airway development, resulting in disruption of proximal–distal patterning. Both loss of function models show that Wnt/β-catenin activity is not associated with nor necessary for epithelial proliferation in the lung. Instead, loss of Wnt/β-catenin signaling results in the specific down-regulation of multiple critical target genes and pathways including N-*myc*, BMP4, and FGF signaling. These data demonstrate that Wnt signaling regulates a molecular hierarchy that promotes distal while repressing proximal airway development, leading to proper patterning of lung epithelium.

Materials and methods

Transgenic mice

The tetO-Dkk1, SP-C/rtTA, tetO-cre, and β-catenin^{flox} mice have been previously described and were maintained on a C57BL/6-CD-1 mixed background (Huelsken et al., 2001; Mucenski et al., 2003). Generation and characterization of the TOPGAL.*lac*Z and BATGAL.*lac*Z Wnt/β-catenin transgenic reporter mice have been previously described (Das-Gupta and Fuchs, 1999; Maretto et al., 2003). Mice were mated with each other, and noon of the day that the vaginal plug was observed was considered E0.5. The pregnant dams were fed food with 1 gm/kg doxycycline starting at E0.5 until embryos were collected at the indicated time points.

Histological methods

Embryos and lung tissue from timed matings of SP-C/rtTA and tetO-Dkk1 mice or SP-C/rtTA:tetO-cre X β-catenin^{flox} mouse crosses were collected and fixed in 4% paraformaldehyde (PFA) for 48 h. Fixed embryos and tissues were dehydrated through a series of increasing ethanol washes and embedded in paraffin. Five-micron sections were generated and dehydrated prior to immunohistochemistry and in situ hybridization. In situ hybridization was performed essentially as described (Morrisey et al., 1996). The SP-A, SP-B, SP-C, CC10, N-*myc*, BMP4, Nkx2.1, and dkk riboprobes have been previously described (Lu et al., 2001; Shu et al., 2002). In situ probes for FGFR1–4 were generated by RT-PCR from E14.5 whole mouse embryo RNA using the following oligonucleotides: FGFR1: sense 5′ acc agc tgt gat gac ctc ac 3′, antisense 5′ tgt aat acg act cac tat agg gcg gcc act ttg gtc aca cgg t 3′; FGFR2: sense 5′ aga agg aga tca cgg ctt cc 3′, antisense 5′ tgt aat acg act cac tat agg gct gcc acg gtg acc gcc tcc 3′; FGFR3: sense 5′ agc tga gga gga gct gat gg 3′, antisense 5′ tgt aat acg act cac tat agg gct gac agg ctt ggc agt acg g 3′; FGFR4: sense 5′ aga cct cac gtg gac aac ag 3′, antisense 5′ tgt aat acg act cac tat agg gct tgg tcg ggc cgg gag gga tc 3′. Immunohistochemistry with the SP-C (Chemicon, 1:200), CC10 (Chemicon,