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Apc deficiency alters pulmonary epithelial cell fate and inhibits Nkx2.1 via triggering TGF-beta signaling

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ABSTRACT

Wnt signaling is critical for cell fate specification and cell differentiation in many organs, but its function in pulmonary neuroendocrine cell (PNEC) differentiation has not been fully addressed. In this study, we examined the role of canonical Wnt signaling by targeting the gene for Adenomatous Polyposis Coli (Apc), which controls Wnt signaling activity via mediating phosphorylation of beta-catenin (Ctnnb). Targeting the *Apc* gene in lung epithelial progenitors by *Nkx2.1-cre* stabilized Ctnnb and activated canonical Wnt signaling. Apc deficiency altered lung epithelial cell fate by inhibiting Clara and ciliated cell differentiation and activating Uchl1, a marker of neuroendocrine cells. Similar to PNEC in normal lung, Uchl1^{positive} cells were innervated. In mice with targeted inactivation of Ctnnb by Nkx2.1-cre, PNEC differentiation was not interrupted. These indicate that, after lung primordium formation, Wnt signaling is not essential for PNEC differentiation; however, its over-activation promotes PNEC features. Interestingly, Nkx2.1 was extinguished in Apc deficient epithelial progenitors before activation of Uchl1. Examination of Nkx2.1 null lungs suggested that early deletion of Nkx2.1 inhibits PNEC differentiation, while late repression does not. Nkx2.1 was specifically inhibited in Apc deficient lungs but not in Ctnnb gain-of-function lungs indicating a functional difference between Apc deletion and Ctnnb stabilization, both of which activate Wnt signaling. Further analysis revealed that Apc deficiency led to increased TGF-beta signaling, which inhibited Nkx2.1 in cultured lung endodermal explants. In contrast, TGF-beta activity was not increased in Ctnnb gain-of-function lungs. Therefore, our studies revealed an important mechanism involving Apc and TGF-beta signaling in regulating the key transcriptional factor, Nkx2.1, for lung epithelial progenitor cell fate determination.

Introduction

Development of the mammalian lung represents a tractable model for analysis of signaling pathways crucial to cell fate determination and differentiation. In the mouse lung development commences around embryonic day 9.5 via outgrowth of anterior foregut endoderm into the surrounding mesenchyme. The enodermally-derived epithelial structure then undergoes repeated branching, controlled by epithelial-mesenchymal interaction, to build the architecture of the lung. During this process, both epithelial cells and mesenchymal cells undergo cell fate determination and differentiation to give rise to what is thought to be more than 40 distinct differentiated pulmonary cell types, specialized in carrying out the function of the mature lung (Morrisey and Hogan, 2010). The earliest epithelial progenitors in the lung express Nkx2.1, a homeodomain transcriptional factor (Minoo et al., 1999). Expression of Nkx2.1 is intimately linked to lung epithelial cell identity. The epithelial progenitors differentiate into multiple types of functionally specialized cells, among which the most abundant in the conducting airways are Clara and ciliated cells, and the most abundant in alveoli are alveolar type 1 and type 2 (AT1 and AT2) cells. The epithelial airways also contain neuroendocrine cells, identified by markers such as Uchl1, Cgrp, & Syp.

Pulmonary neuroendocrine cells (PNECs) are essential for lung function. Disruption of PNEC differentiation results in neonatal death due to respiratory failure (Borges et al., 1997). PNEC

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hyperplasia is observed in several pediatric lung diseases including Bronchopulmonary Dysplasia (BPD), Sudden Infant Death Syndrome (SIDS) and Congenital Central Hypoventilation Syndrome (CCHS) (Cutz et al., 2007a, 2007b). To date, it is unclear whether PNECs originate from neural crest or from the same progenitor as other cells that comprise the airway epithelium (Ito et al., 1997). Lineage-tracing studies have shown that some PNECs are differentiated from ID2 expressing epithelial cells, whereas none of the PNECs seem to originate from epithelial cells expressing a 3.7 kb human Spc promoter (Perl et al., 2002; Rawlins et al., 2009). It has been reported that Mash1 is required for PNEC differentiation (Ito et al., 2000).

Wnt signaling, mediated by beta-catenin (Ctnnb), is critical for normal organogenesis, stem cell renewal and tumorigenesis (Ling et al., 2009; Logan and Nusse, 2004; van Amerongen and Berns, 2006). The activity of Ctnnb is regulated by a protein complex, a major component of which is Adenomatous Polyposis Coli or Apc, a 310-kDa multifunctional protein (Polakis, 1997). In the absence of Wnt ligands, Ctnnb is phosphorylated at the destruction complex composed of Apc, Axin, and Gsk3beta, and is subsequently ubiquitinated and degraded. Deletion of Apc disrupts the destruction complex, which results in Ctnnb stabilization and activation of canonical Wnt signaling. Mutations of Apc or Ctnnb are frequently observed in patients with colorectal cancer. Experimentally, two approaches have been developed to hyper-activate canonical Wnt signaling in mice. These are deletion of Ctnnb exon 3 (Ctnnb gain-of-function) and deletion of Apc. Ctnnb exon 3 is the site of phosphorylation targets (S33, S37, T41, S45) that lead to its degradation. Both models have been used extensively and it is generally thought that the two approaches have similar functional consequences. (Harada et al., 1999; Kuraguchi et al., 2006; Oshima et al., 1995).

Functions of Ctnnb in the lung have been studied and found to be closely linked to cell fate specification (De Langhe and Reynolds, 2008). In gain-of-function studies, deletion of Ctnnb exon 3 by Ccsp-cre which targets mostly Clara cells leads to expansion of a lung stem cell pool. Using Nkx2.1-cre which targets epithelial progenitors early in lung morphogenesis, deletion of Ctnnb exon 3 was shown to result in cell fate changes that favored neuroendocrine features (Li et al., 2009). Deletion of Ctnnb exon 3 by Spc-cre which targets distal lung endoderm led to loss of bronchiolar epithelium and distal airway ectasis, a phenotype

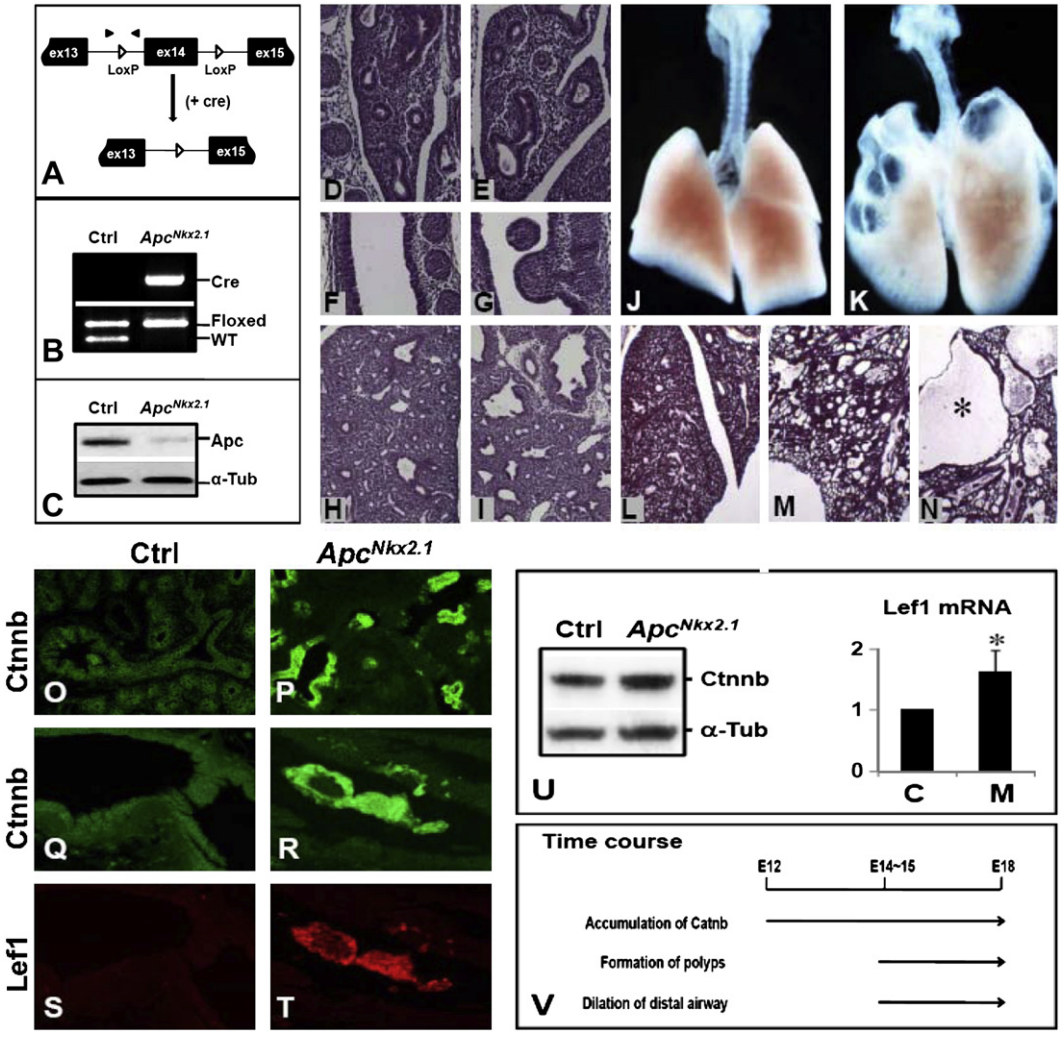

Fig. 1. (A) Illustration of exon 14 of the Apc gene that is flanked by the LoxP sequences. (B) Genotyping of $Apc^{Nkx2.1}$ embryos. (C) Western blot analysis shows that levels of Apc were decreased in $Apc^{Nkx2.1}$ lungs. (D and E) Histology of E12 control and $Apc^{Nkx2.1}$ lungs. (F–I) Histology of E15 control and $Apc^{Nkx2.1}$ trachea (F and G) and lungs (H and I). (J and K) Gross morphology of E18 control and $Apc^{Nkx2.1}$ lungs. (L–N) Histology of E18 control (L) and $Apc^{Nkx2.1}$ lungs (M and N). Panel N shows the severely dilated airspace (*). (O and P) Immunofluorescent staining of Ctnnb in control and $Apc^{Nkx2.1}$ lungs. (Q–T) Co-immunofluorescent staining of Ctnnb (green) and Lef1 (red) in control (Q and S) and $Apc^{Nkx2.1}$ (R and T) main-stem-bronchi (MSB) indicates increased Lef1 expression in epithelial cells with Ctnnb accumulation. (U) Increases of Ctnnb were shown by western blot analysis. Increases of Lef1 mRNA were shown by realtime PCR analysis. Asterisk indicates $p < 0.05$. (V) Illustration of the time course of Ctnnb accumulation, polyp formation and airspace dilation in the $Apc^{Nkx2.1}$ lungs.

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