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Dlg5 maintains apical aPKC and regulates progenitor differentiation during lung morphogenesis

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ABSTRACT

Cell polarity plays an important role in tissue morphogenesis; however, the mechanisms of polarity and their role in mammalian development are still poorly understood. We show here that membrane-associated guanylate kinase protein Dlg5 is required for proper branching morphogenesis and progenitor differentiation in mammalian lung. We found that during lung development Dlg5 functions as an apical–basal polarity protein, which is necessary for the apical maintenance of atypical protein kinase C (aPKC). These results identify Dlg5 as a regulator of apical polarity complexes and uncover the critical function of Dlg5 in branching morphogenesis and differentiation of lung progenitor cells.

Introduction

Cell polarity is important for proper morphogenesis of all mammalian organisms; however, the mechanisms of cell polarity and, even more importantly, the particular role and significance of these mechanisms in the major developmental events are still poorly understood. Significant knowledge about the proteins involved in apical–basal cell polarity was generated using such model organisms as *Caenorhabditis elegans* and *Drosophila* (McCaffrey and Macara, 2012; Wodarz and Nathke, 2007). These studies identified atypical PKC (aPKC)/Par3/Par6 proteins as critical members of the apical cell polarity machinery, which localize to the apical membrane domain and are necessary for the establishment and maintenance of the apical membrane domain identity (McCaffrey and Macara, 2009b). In contrast, the Par1, Par4, Dlg, Lgl and Scribble proteins localize to the basolateral membrane domain and are required for basolateral domain formation and maintenance (Yamanaka and Ohno, 2008). In general, the function and the mechanisms of the apical membrane polarity complexes aPKC/Par6/Par3 are understood much better than the function and the mechanisms of the basolateral polarity proteins. Par3 and Par6 are the PDZ (PSD95/Dlg/ZO1) domain-containing molecular adapter and scaffold proteins, which bind to aPKC, the only enzyme in the apical polarity complex (McCaffrey and Macara, 2009b). aPKC phosphorylates and negatively regulates the function of Par1 and Lgl basolateral polarity proteins (Betschinger et al., 2003; Hurov et al., 2004). Reciprocally, Par1 phosphorylates and negatively regulates the membrane association and cell polarity function of Par3 (Benton and St. Johnston, 2003). *Dlg* is an essential basolateral polarity gene, which genetically interacts with Lgl and Scribble in Drosophila (Bilder et al., 2000; Woods and Bryant, 1991). Dlg is a member of the Membrane Associated Guanylate Kinase (MAGUK) proteins. The functional role of Dlg in the regulation of cell polarity remains obscure; however, MAGUK proteins usually function as protein scaffolds that help to cluster multiple transmembrane and accessory proteins to hold together the elements of individual signaling pathways, and it is likely that Dlg performs similar function at the lateral membrane domain (Yamanaka and Ohno, 2008).

Dlg5 is conserved throughout the Metazoan evolution gene that differs from the Drosophila *dlg* and mammalian *Dlg1-4* because in addition to guanylate kinase and PDZ domains, it contains N-terminal CARD and coiled coil domains (Nechiporuk et al., 2007). Function of *Dlg5* in Drosophila has not been investigated. Polymorphism in human Dlg5 protein sequence is associated with predisposition to the Crohn's disease: however,

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the mechanisms of Dlg5 in Crohn's disease are not well understood (Stoll et al., 2004). In renal and mammary epithelial cell lines, knockdown of Dlg5 activates cell migration and promotes TGF-β-mediated epithelial–mesenchymal transition (Sezaki et al., 2012; Smolen et al., 2010). To determine the physiological function of Dlg5 in mammalian organism, we have previously generated and analyzed *Dlg5*$^{-/-}$ mice (Nechiporuk et al., 2007). We found that *Dlg5*$^{-/-}$ mice develop brain hydrocephalus and kidney cysts. Biochemical analysis revealed an important function of Dlg5 in facilitating the delivery of N-cadherin to the plasma membrane (Nechiporuk et al., 2007).

In this study we analyzed the role of Dlg5 in developing mammalian lung. The mammalian lung is one of the best-studied examples of a developing organ that undergoes the highly coordinated process of branching morphogenesis coupled with timely progenitor cell differentiation. Together, these events result in the formation of an organ containing branched airways that terminate in millions of functional alveolar sacs enabling adequate lung function (Metzger et al., 2008). Failure of proper lung development can result in neonatal death or chronic pulmonary disease, which is often associated with the enlargement of peripheral airspaces (Bourbon et al., 2009; Snider, 1992). We show here that Dlg5 is required for proper mammalian lung morphogenesis as *Dlg5*$^{-/-}$ mice display abnormal branching morphogenesis and differentiation of lung progenitor cells and develop completely penetrant lung airspace enlargement and emphysema-like phenotype. We demonstrate that *Dlg5*$^{-/-}$ lung epithelial cells display prominent apical–basal polarity defects, which may be responsible for the defects in branching and differentiation.

Results

Failure of normal lung morphogenesis and emphysema-like phenotype in Dlg5$^{-/-}$ *mice.*

We previously reported that approximately half of the *Dlg5*$^{-/-}$ mice die perinatally (Nechiporuk et al., 2007). The analysis of the surviving *Dlg5*$^{-/-}$ adults revealed prominent and completely penetrant lung abnormalities. Therefore, here we specifically focused on the analysis of the role of Dlg5 in murine lung morphogenesis. Histological examination of adult lungs demonstrated an emphysema-like phenotype in *Dlg5*$^{-/-}$ mice with prominent dilatation of the distal airspaces and an overall decrease in number of alveolar septa (Fig. 1A–B'). To assess the origin of these morphological defects, we performed macroscopic and histological analyses of the lungs from *Dlg5*$^{-/-}$ and wild-type mice at different times of postnatal development. Similar to adult *Dlg5*$^{-/-}$ animals, newborn *Dlg5*$^{-/-}$ pups displayed enlarged distal airspaces that contained few alveolar septa and presented with areas of collapsed lung parenchyma (Fig. 1C–D', arrows). The macroscopic analyses of 1-day-old (P1) lungs also revealed the prominent enlargement of distal airspaces in *Dlg5*$^{-/-}$ pups (Fig. 1E–F').

Since lung defects were already present in newborn mutants, we histologically examined the lungs of *Dlg5*$^{-/-}$ and wild-type mice at different times during embryonic development. We found that the lung branching pattern was indistinguishable between wild-type and *Dlg5*$^{-/-}$ embryos at E12.5. However, starting from E13.5 and throughout the subsequent embryogenesis, 100% *Dlg5*$^{-/-}$ lungs showed a significant decrease in the number, accompanied by a prominent increase in the size of terminal tubules within the developing lung (Fig. 1G–K'). We conclude that the initial onset of lung abnormalities in *Dlg5*$^{-/-}$ mice occurs between E12.5 and E13.5 of development.

Dlg5 is required for lung branching morphogenesis

Between days E9.5 and E16.5 of lung development (pseudoglandular stage) primary buds undergo the highly coordinated process of branching morphogenesis that results in the formation of tree-like structures that end with multiple terminal tubules (Morrisey and Hogan, 2010). The histological abnormalities observed in the *Dlg5*$^{-/-}$ lungs were consistent with a potential defect in branching morphogenesis. To study branching, we analyzed fixed lungs using macroscopic examination and whole-organ immunostaining with anti-E-cadherin antibodies, which facilitates 3-dimensional visualization of the bronchial tree (Metzger et al., 2008). While the comparison of lungs from the *Dlg5*$^{-/-}$ and wildtype littermate embryos revealed little difference at E12 and E12.5, prominent defects in branching morphogenesis were observed in *Dlg5*$^{-/-}$ lungs at E13.5 (Fig. 2A–D'). E13.5 *Dlg5*$^{-/-}$ lungs display delay in branching, with an overall decrease in the number of branches and a prominent dilation of distal tubules (Fig. 2C–D'). Lung branching morphogenesis in the developing mouse embryo is a highly stereotypical process that is comprised of 3 branching modes: domain branching and planar and orthogonal bifurcation of terminal buds (Metzger et al., 2008). Comparison of left lung lobes in E13.5 wildtype and *Dlg5*$^{-/-}$ littermates show a similar number of L branches (L1–L5); however, all terminal buds are bifurcated (a–p) in the wildtype, but not in the *Dlg5*$^{-/-}$ lungs (Fig. 2D–E, $n=4$). Moreover, while the domain branching mode results in the formation of D branches in both wildtype and *Dlg5*$^{-/-}$ lungs, the d3 branch is missing in the high order L1 and L2 branches of the *Dlg5*$^{-/-}$ embryos (Fig. 2D, D'). The decrease in the number of branches and the dilation of terminal buds are consistent with the histological phenotypes observed in E13.5–E15.5 *Dlg5*$^{-/-}$ lungs (Fig. 1H–I'). We conclude that Dlg5 is required for proper lung branching morphogenesis and that the absence of Dlg5 results in an overall defect in branching with dilation of the distal tubules and terminal buds.

Epithelial–mesodermal interactions play an important role in the regulation of lung development and defects in either epithelial or mesenchymal tissues can result in an abnormal branching morphogenesis (Morrisey and Hogan, 2010). To determine whether *Dlg5* is expressed in epithelial or mesodermal compartments during lung development, we analyzed *Dlg5* expression using in situ hybridization. While *Dlg5* was expressed throughout the lung tissue, the highest levels of *Dlg5* expression were present in the epithelial tubes, the same structures that fail to branch and instead dilate in *Dlg5*$^{-/-}$ embryos (Fig. 2F–G).

Several signal transduction pathways including Wnt, FGF, PDGF and BMP signalings have been implicated in the regulation of lung branching morphogenesis (Cardoso and Lu, 2006; De Langhe and Reynolds, 2008). We therefore analyzed whether Wnt, FGF, PDGF, or BMP signalings are perturbed in *Dlg5*$^{-/-}$ lungs. Beta-catenin is pivotal for canonical Wnt signaling and Dlg5 can physically interact with β-catenin (Wakabayashi et al., 2003). To determine whether canonical Wnt signaling is affected in *Dlg5*$^{-/-}$ lungs, we crossed our mutants with TOPGAL mice carrying LacZ reporter for β-catenin (DasGupta and Fuchs, 1999). Staining for LacZ activity did not reveal significant differences between the control and *Dlg5*$^{-/-}$ lungs (Supplementary Fig. 1). Similar to Wnt pathway, no significant differences in the levels of phosphorylated FGFR2, PDGFα, or their downstream target, ERK1/2, were found between wild-type and *Dlg5*$^{-/-}$ lungs (Supplementary Fig. 2A–C). In addition, we did not find significant differences in the phosphorylation of SMAD1/5, a downstream target and transducer of BMP signaling (Supplementary Fig. 2D). In situ hybridizations revealed overall similar levels of Fgf10 mRNA, although we noted that the Fgf10 expression pattern