Which End Is Up? Defining Cell Polarity During Organogenesis

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Organ development is a carefully scripted process of cellular proliferation, differentiation, and apoptosis. Persistently defining cell polarity by identifying the apical (top of the cell) and basolateral (bottom and sides of the cell) surfaces is crucial for the proper development of organs, and defects in this system can result in severe developmental abnormalities and neonatal death. While several of the key proteins defining cell polarity have been identified, the role these proteins play during development is still often unclear. In a recent report published in *Developmental Biology*, Drs. Tamilla Nechiporuk and Valeri Vasioukhin (Division of Human Biology) show that Dlg5 regulates complexes defining apical polarity and is critical for lung development.

Several key markers of cell polarity have been defined in model species; atypical PKC (aPKC), Par3, and Par6 define apical cell polarity, while Par1, Par4, Dlg, Lgl, and Scribble localize to the basolateral cell surface. Dlg proteins are members of the Membrane Associated Guanylate Kinase (MAGUK) family of proteins, and are essential basolateral polarity proteins. MAGUK proteins generally act as scaffolds to coordinate the localization of proteins involved in signaling pathways. The Vasioukhin laboratory previously reported several developmental abnormalities in *dlg5*-/- mice, including hydrocephaly, kidney cysts, and emphysema-like lung abnormalities. All surviving mice shared the lung abnormalities, the team focused their efforts on this tissue.

Mouse gestation lasts three weeks, and fetal lung development proceeds through a highly coordinated process. During the earliest phases the major airways and bronchial tree develop. Next, epithelial differentiation and formation of the air-blood barrier occur, and finally the air spaces expand.

Newborn *dlg5*-/-pups already had enlarged airspaces and areas of collapsed lung, suggesting the abnormality occurred during embryonic development. The team found that after 12.5 days of development *dlg5*-/- and wildtype embryos were indistinguishable; however, after 13.5 days of development*dlg5*-/- pups had fewer and larger terminal tubules compared to wildtype embryos. The highest concentrations of Dlg5, by in situ hybridization, localized to the epithelial tubes, which had failed to branch in *dlg5*-/- animals. These defects were also accompanied by morphological changes in the developing lung epithelium. Alveolar epithelium type 1 cells (AEC1), which differentiate into flat

cells lining the surface of the alveoli, were underrepresented in *dlg5*-/- embryos, and columnar cells with basally localized nuclei were replaced with cuboidal cells containing centrally localized nuclei.

Immunostaining and Western blot analyses confirmed that early differentiation of lung progenitor cells was normal; however, the team found that T1α, aPKC, and ezrin-radixin-moesin (ERM) proteins did not properly localize to the apical surface of *dlg5*-/- cells, while other apical proteins, such as Par3 and Par6b, were appropriately positioned. Immunohistochemistry demonstrated that Dlg5 localizes to both apical and basal surfaces, and at the apical surface it co-localizes with aPKC. Because aPKC is the only kinase among the apical proteins, the team asked whether or not this altered localization also changed aPKC enzymatic activity. Western blots showed equivalent levels of activated phosphor-sPKC, suggesting that Dlg5 is necessary for the localization, but not the activation, of aPKC.

"The major defining point of our study was the finding that cell polarity was disrupted in Dlg5-null lung epithelial cells and this is the earliest abnormality that we can find in the developing lungs in Dlg5 mutant embryos. An important question that remains unanswered is the molecular mechanism connecting Dlg5 with the maintenance of cellular polarity. This will be our main research direction in the future," said Dr. Vasioukhin.

Nechiporuk T, Klezovitch O, Nguyen L, Vasioukhin V. 2013. Dlg5 maintains apical aPKC and regulates progenitor differentiation during lung morphogenesis. *Dev Biol.* Epub ahead of print, doi: 10.1016/j.ydbio.2013.02.019.

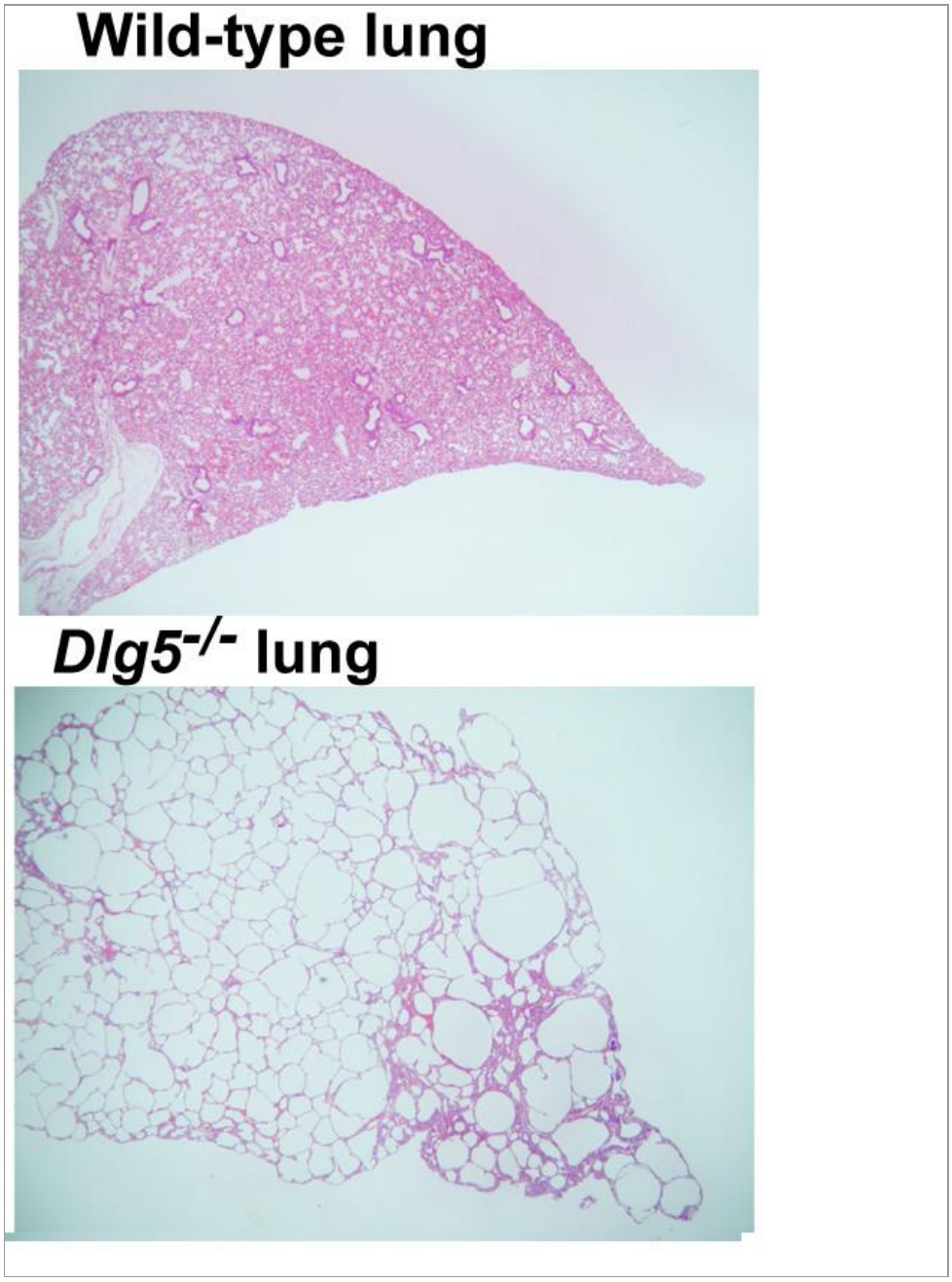

Image courtesy Valeri Vasioukhin

Cross-section lung from wild-type (top) and dlg5-/- lung. There are fewer and larger airspaces in the dlg5-/- lung relative to wild-type animals.