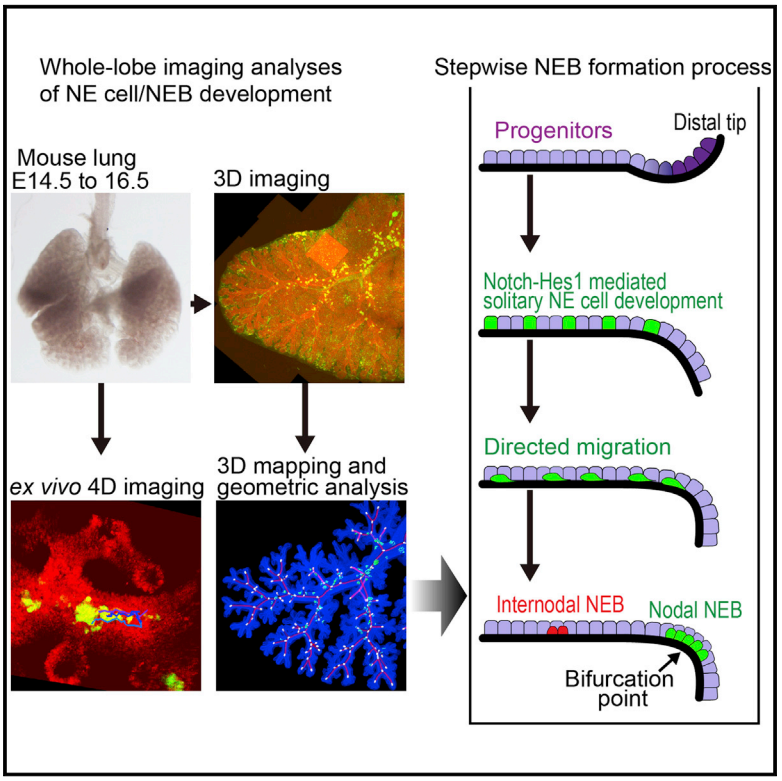
Report

Cell Reports

Directed Migration of Pulmonary Neuroendocrine Cells toward Airway Branches Organizes the Stereotypic Location of Neuroepithelial Bodies

Graphical Abstract



Highlights

- We established methods for 3D mapping and ex vivo 4D imaging of developing lungs
- Nodal, but not internodal, NEBs stereotypically localize at the bifurcation area
- Notch-Hes1 signaling creates solitary NE cells and regulates the NE cell population
- Solitary NE cells migrate to bifurcation points to form stereotypic nodal NEBs

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In Brief

Noguchi et al. developed methods of 3D mapping and 4D live imaging of developing lung epithelial cells. The authors discovered that neuroepithelial body (NEB) development proceeds in a stepwise manner in which solitary neuroendocrine cells differentiate and subsequently undergo directional migration toward a stereotypic position of branching airways to organize NEBs.

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Directed Migration of Pulmonary Neuroendocrine Cells toward Airway Branches Organizes the Stereotypic Location of Neuroepithelial Bodies

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SUMMARY

The airway epithelium consists of diverse cell types, including neuroendocrine (NE) cells. These cells are thought to function as chemoreceptors and as a component of the stem cell niche as well as the cells of origin in small-cell lung cancer. NE cells often localize at bifurcation points of airway tubes, forming small clusters called neuroepithelial bodies (NEBs). To investigate NEB development, we established methods for 3D mapping and ex vivo 4D imaging of developing lungs. We found that NEBs localize at stereotypic positions in the bifurcation area irrespective of variations in size. Notch-Hes1 signaling contributes to the differentiation of solitary NE cells, regulating their number but not localization. Live imaging revealed that individual NE cells migrate distally to and cluster at bifurcation points, driving NEB formation. We propose that NEB development is a multistep process involving differentiation of individual NE cells and their directional migration to organize NEBs.

INTRODUCTION

The epithelium of the mammalian lung consists of various cell types that serve to support respiratory function (Morrisey and Hogan, 2010; Rackley and Stripp, 2012). The distribution patterns of these cells are determined at the pseudoglandular stage (embryonic days [E]11.5–16.5 in the mouse) of the fetal lung, during which airway branching morphogenesis occurs and the terminal buds come to contain a population of multipotent epithelial progenitors (Perl et al., 2002; Rawlins et al., 2009a). As the bronchial tree extends, descendants of the multipotent progenitor cells give rise to lineage-restricted progenitors that produce all of the differentiated cells. One such cell type, neuroendocrine (NE) cells, are bi-functional epithelial cells that are detected either as scattered solitary cells or as small clusters in the trachea to bronchioles but not the alveoli (Cutz et al., 1978; Fujita et al., 1998; Linnoila, 2006). These NE cell clusters are referred to as neuroepithelial bodies (NEBs), and multiple reports have shown that NEBs are frequently observed at bifurcation points of branching airways. These are referred to as "nodal" NEBs (Avadhanam et al., 1997; Cutz et al., 1978; Hoyt et al., 1990; Figure 1A), whereas NEBs in inter-bifurcation regions are called "internodal." This unique positioning of NEBs may be beneficial for their ability to sense hypoxic conditions through the chemoreceptor activity of NE cells (Linnoila, 2006; Youngson et al., 1993) because inspired air is trapped at bifurcation points. Mature NEBs are innervated on the basal side by vagal nerve afferents (Adriaensen et al., 1998; Chang et al., 2015). Aberrant large clusters of NE cells have been observed in neuroendocrine hyperplasia of infancy (NEHI) (Deterding et al., 2005; Young et al., 2011) and neuroendocrine tumors, including small-cell lung cancer (Semenova et al., 2015), highlighting the importance of the regulation of NE cell populations to human health. Involvement of NEBs in adult tissue regeneration has also been investigated. In a naphthalene-induced club cell injury model, epithelial tissue regeneration occurs preferentially around nodal NEBs at bifurcation points, suggesting that NEBs may serve as a microenvironment that stimulates variant club cells as progenitors (Hong et al., 2001; Reynolds et al., 2000). However, we note that epithelial regeneration can occur in the absence of NEBs because of the redundant function of regular Club cells as epithelial progenitors (Rawlins et al., 2009b; Song et al., 2012).

Despite the functional importance of NE cells and NEBs, their developmental course remains unclear, in part because of technical limitations in obtaining high-resolution images and quantitative analysis of the behavior of epithelial cells in the context of a 3D branching morphology. In this study, we established methods for 3D mapping of lung epithelial cells and ex vivo 4D imaging of the developing lung. Using these methods, we provide evidence suggesting a stepwise development of NEBs, involving the differentiation of solitary NE cells and their directional migration toward bifurcation points to form NEBs following branching morphogenesis.

RESULTS

3D Mapping and Quantification of the NEBs on Branching Airways

Immunohistochemical analyses have detected nodal NEBs at airway bifurcation structures but are insufficient to determine