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Cell polarity and spindle orientation in the distal epithelium of embryonic lung

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Abstract

A proper balance between self-renewal and differentiation of lung specific progenitors at the distal epithelial tips is absolutely required for normal lung morphogenesis. Cell polarity and mitotic spindle orientation play a critical role in the self-renewal/differentiation of epithelial cells and can impact normal physiological processes, including epithelial tissue branching and differentiation. Therefore, understanding the behavior of lung distal epithelial progenitors could identify innovative solutions to restoring normal lung morphogenesis. Yet little is known about cell polarity, spindle orientation and segregation of cell fate determinant in the embryonic lung epithelium, which contains progenitor cells. Herein, we provide the first evidence that embryonic lung distal epithelium is polarized and highly mitotic with characteristic perpendicular cell divisions. Consistent with these findings, mInsc, LGN and NuMA polarity proteins, which control spindle orientation, are asymmetrically localized in mitotic distal epithelial progenitors of embryonic lungs. Furthermore, the cell fate determinant Numb is asymmetrically distributed at the apical side of distal epithelial progenitors and segregated to one daughter cell in most mitotic cells. These findings provide evidence for polarity in distal epithelial progenitors of embryonic lungs and provide a framework for future translationally oriented studies in this area.

Keywords

lung progenitors; progenitor cell behavior; cell polarity; cell fate

Introduction

Cell polarity is identified by asymmetry in the distribution of cellular constituents within a single cell. It is crucial for various cellular processes including cell specification and migration as well as asymmetric division. Cell polarity plays a fundamental role in helping to organize and integrate complex molecular signals so that cells can make decisions concerning fate, orientation, proliferation, differentiation, and interaction (Wodarz, 2002; Nelson, 2003). In the mature lung, the single layer of epithelial cells, which line all distal air sacs, forms the crucial barrier between the air and the interior of the organism, and its correct functioning is essential to life. To perform this vital function, each epithelial cell is polarized, which is also essential for fetal lung epithelial cells to grow properly (Matsui et al., 1999). Yet, despite their clear importance, cell polarity and mitotic spindle orientation remain largely uncharacterized in embryonic distal lung epithelium, which represents the

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epithelial progenitor pool that expresses Sox9, Id2 and N-myc (Liu and Hogan, 2002; Okubo et al., 2005; Rawlins, 2008; Rawlins et al., 2009). Herein we report on cell polarity and spindle orientation in progenitor cells at the distal epithelial tips of E14-14.5 murine lungs.

Results and Discussion

In different organs, epithelial cells characteristically show apical–basal polarity, which is necessary for their function as barriers between different extracellular environments (Drubin and Nelson, 1996; Mostov et al., 2000). It is well established that actin and actin-associated proteins are essential for generating molecular and morphological cell polarity (Winder and Ayscough, 2005). In distal lung epithelium, as in other epithelial cell types, f-actin myosin II-b, β-catenin and zonula occludens protein (ZO-1) localize predominantly to the apical junctional complex, which is essential for cell polarization (Tepass, 2003; Fig. 1A, B, and data not shown). Similar asymmetric apical localization has been seen for E-cadherin as well as for pericentrin, a conserved centrosome protein involved in microtubule organization that is located apically in epithelial cells (Fig. 1C,D; Doxsey et al. 1994; Chenn et al. 1998), and also for polarity proteins Par-3 and Par-6 (Fig. 1E,F). These data suggest that distal embryonic lung epithelial cells are indeed polarized.

We next addressed whether distal embryonic lung epithelial cells divide perpendicular to the basement membrane or laterally within the plane of the epithelium. At the pseudoglandular stage, staining with tubulin, an indicator of cells in metaphase (Daar et al., 1991), and PCNA antibodies showed that distal epithelial cells are indeed highly proliferative/mitotic (Fig. 2A,B). Most of these mitotic cells were apparently dividing perpendicularly to the basement membrane, as judged by staining with anti-tubulin, which localized at apical and basal sides of mitotic cells (Fig. 2 C,D; arrowheads).

To facilitate quantification of cells dividing perpendicularly versus laterally, we first stained distal epithelium for centrosomes with pericentrin antibody. Then, mitotic cells were quantified based on centrosome orientation relative to the collagen IV-stained basement membrane, in order to distinguish parallel (lateral) from perpendicular spindle alignments in mitotic cells (Figs. 1C, 2E-G). Centrosomes that were oriented at 0 ± 30° to the basement membrane were classed as parallel; those that were oriented at 90 ± 30° were classed as perpendicular. In E14-14.5 distal epithelium, most cell divisions (82%) occurred perpendicular to the basement membrane, while the remaining 18% of mitotic cells had an alignment that appeared parallel (Fig. 2G, 82.0 ± 3.0% vs. 18.0 ± 2.0%, respectively; $n = 3$).

The regulation of the spindle orientation and cell division axis, which can impact normal physiological processes, including epithelial tissue branching and differentiation is often associated with cell polarity regulation in polarized cells in model organisms (reviewed in Betschinger and Knoblich, 2004). The orientation and positioning of the mitotic spindle, which determines the plane of cell division, is tightly regulated in polarized cells such as epithelial cells by intrinsic and extrinsic cues. These cues include certain cell polarity regulating asymmetrically localized cortical proteins, cell–cell adhesion, and cell-matrix adhesion. Therefore, to further distinguish parallel from perpendicular spindle alignments, and to distinguish how distal epithelial cells control their spindle orientation, we next determined the expression pattern of asymmetrically localized cortical proteins, which have spindle orientation-regulatory functions (Fig. 3).

In Drosophila, chick and mammalian epithelium, a protein complex containing Inscuteable, LGN and NuMA captures a spindle pole at the apical cortex, aligning the spindle with the apical–basal axis and thus regulating cell fate (Kraut et al., 1996; Du et al., 2001; Roegiers and Jan, 2004; Lechler and Fuchs, 2005; Žigman et al., 2005; Morin et al., 2007).

Immunofluorescence and Western blot showed that the expression of mouse Inscuteable (mInsc), LGN, NuMA and the cell fate determinant Numb is conserved in distal lung epithelium (Fig. 3A-E). In the mammalian epithelium, LGN, NuMA and mInsc proteins localize to the apical side of cells only during apical-basal (perpendicular) cell division, and have a diffuse localization in cells that are in interphase or that are undergoing lateral divisions (Lechler and Fuchs, 2005). As expected, apical localization of LGN, mInsc and NuMA was seen at the cortex of most mitotic cells, and accounted for more than 80% of all mitoses in distal embryonic lung epithelial cells (Fig. 3A-C,F; $n = 3$), which further suggests that most of these cells divide perpendicularly.

In epithelial and other cell types, the axis of polarity that will determine the orientation of the apical-basal cell division plane is defined by cell fate determinants (CFDs), such as Numb and Par-proteins. Intrinsic CFDs are asymmetrically localized in dividing cells, and preferentially segregate into one of two sibling daughter cells in order to mediate asymmetric cell divisions (Betschinger and Knoblich, 2004). We found that the cell fate determinant Numb had a polarized asymmetric localization at the apical side of distal epithelial cells with little or no staining at the basal pole (Fig. 3D). Closer inspection of mitotic cells revealed that Numb staining is consistently concentrated as a crescent at the apical pole of one daughter cell (apical daughter) in 88± 5.0% of wildtype distal epithelial tip cells (Fig. 3G, $n = 3$). Thus, the more perpendicular a cell division is (Figs. 2, 3), the more likely it is to segregate Numb preferentially to one daughter cell in mitotic lung epithelial cells.

In this brief study, to our knowledge, we provide the first evidence that embryonic distal lung epithelium is polarized with characteristically perpendicular divisions. We also demonstrate for the first time that the expression of the polarity proteins mInsc, LGN and NuMA, which control mitotic spindle orientation (Siller and Doe, 2009; Zheng et al., 2010), are conserved in embryonic distal lung epithelial cells. In addition, we show that the cell fate determinant Numb is asymmetrically localized/segregated in mitotic distal epithelium

Alveolar hypoplasia, which characterizes bronchopulmonary dysplasia in extremely premature human infants, wherein a significant deficiency of stem/ progenitor cells probably occurs, are common features of human prematurity and/or lung injury and are thus of major public health concern. Cell polarity is critical for the proper balance between self-renewal and differentiation of lung specific progenitors, which is absolutely required for normal lung morphogenesis and regeneration. Moreover, loss of epithelial cell polarity is involved in several lung diseases, including lung epithelial cancers and Chronic Obstructive Pulmonary Disease, which are likewise related to disruption of lung epithelial differentiation and cellular function (Xu et al., 2006). Therefore, understanding the behavior of lung epithelial progenitors localized in the distal embryonic lung epithelium, as described herein, could identify innovative solutions to restoring normal lung morphogenesis and possibly regeneration of the gas diffusion surface. Our findings now provide a framework for future translationally orientated studies in this area.

Brief Experimental Procedures

In this study, E14-14.5 was used as the developmental stage of choice to analyze the behavior of distal embryonic lung epithelium because cell proliferation is relatively high. Immunohistochemistry was performed in triplicate. Briefly, lungs were fixed in 4% paraformaldehyde, dehydrated in ethanol, impregnated with toluene, embedded in paraffin, and processed into serial paraffin sections using standard procedures. Sections were processed for antigen retrieval by boiling the sample for 12 minutes in Na-citrate buffer (10mM, pH 6.0; Vector), then blocked and stained with antibodies described in

Supplemental Table 1 using standard immunohistochemistry procedures. Western blot and counting cell number were performed as described (delMoral et al. 2006a,b, El-Hashash et al., 2010 in press).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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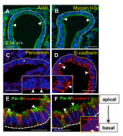

Figure 1. Lung distal epithelium is polarized

Immunofluorescence at E14 shows strong signals for actin (A), myosin II-b (B), pericentrin (C), E-cadherin (D), Par-3 (E) and Par-6 (F) proteins at the apical side of distal epithelial cells (arrowheads). Dashed line represents the collagen IV-stained basement membrane. Insets in C,D represent the area marked with an asterisk in the same panel. Inset in F shows immunofluorescence staining of E14 basement membrane (arrows) with collagen IV antibody. Scale bars: 50 μm.

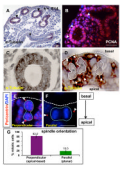

Figure 2. Lung distal epithelium is mitotic with perpendicular cell divisions

(A-D) Antibody staining at E14 shows strong signals for α-tubulin and PCNA in distal (arrowheads) rather than proximal (arrow; A) epithelium. (D) represents electronic magnification of boxed area in C, and shows perpendicular/apical-basal (arrowheads) spindle alignments relative to collagen IV-stained basement membrane (dashed line) in mitotic cells (a,b,c). (E-F) Most mitotic cells in the distal epithelium divide perpendicularly, as represented by the perpendicular orientation of pericentrin-stained centrosomes (arrowheads, arrows) relative to the basement membrane (dashed line; E,F), while only a few mitotic cells have their centrosomes aligned parallel to the basement membrane (G; arrowheads). (G) Quantitation of spindle orientations, expressed as a percentage of all divisions in the distal epithelium from the experiments shown in E-F (*n*=48). Scale bars: 50 μm.

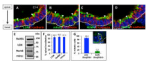

Figure 3. Expression of the polarity proteins LGN, mInsc, NuMA and Numb in lung distal epithelium

as shown by immunofluorescence (A-D) and Western blot (E). Note polarized apical localization of these proteins (A-D; arrowheads) relative to collagen IV-stained basement membrane (dashed line). (F) Quantitation of the apical localization of proteins shown in A-C, which is expressed as a percentage of all cells in the distal epithelium (*n*=87). (G) Quantification of late mitotic distal epithelial cells, with Numb inherited by one (inset in G, which represents the area marked with an asterisk in D) or both daughter cells at E14. This is expressed as a percentage of all divisions in the distal epithelium (*n*=42). Scale bars: 50 μm.