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Lung development: AT1 and AT2 property

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Abstract: The human respiratory system consists of the upper and the lower respiratory tracts. Anatomically, the lower respiratory tract consists of the trachea, bronchi, bronchioles (terminal bronchioles and respiratory bronchioles), alveolar duct, alveolar duct sacs, and alveoli. Alveoli are composed of two epithelial cell types, cuboidal alveolar type 2 (AT2) cells that secrete surfactant to prevent alveolar collapse and function as stem cells to regenerate alveolar type 1 (AT1) cells during damage repair, and squamous AT1 cells that cover most of the surface area of the alveoli and mediate gas exchange. Previous studies mainly focused on AT2 cells; this review summarizes the current studies on lung development and property of AT1 cells.

Introduction

The mammalian respiratory system is a tree-like architecture consisting of a single trachea, two bronchi, thousands of bronchioles and millions of alveoli (Chen *et al.,* 2018). The lungs contain about 300 million alveoli, which comprise about 160 cm^2 of surface area for air exchange (El-Hashash, 2018; Rosen *et al*., 2015). Lung development is composed of three consecutive periods: the embryonic period, fetal period and postnatal period (Schittny, 2017). During the ebryonic period, lung organogenesis is mainly completed. The fetal period further divides into pseudoglandular, canalicular, and saccular stages. The postnatal period mainly contains alveolar formation and microvascular network maturation (Schittny, 2017) (Fig. 1(A)).

Lung Development

Human lung development begins at approximately day 28 of gestation and originates from anterior foregut endoderm cells (Herriges and Morrisey, 2014). The primitive germ layer of the early embryo firstly differentiates to form the left and right primitive bronchial buds, and the left primary bronchial buds form two secondary bronchial buds, the right primary bronchial buds develop into three secondary bronchial buds at the 5th week of gestation, then the left and right secondary bronchi buds develop into corresponding pulmonary lobes (El-Hashash, 2018). The lungs begin to take shape at 6-16 postconceptional weeks (PCW), to further increase and branch at 16-26 PCW, at the same time accompanying the bronchioles and alveolar ducts formation and the vascularization of lung tissue. Finally, alveolar formation and separation begin at 20 PCW, until several years after birth (Schittny, 2017; Warburton *et al*., 2010a).

Lung development is actually the result of the interaction between mesenchymal and epithelial cells, and its development process is strictly regulated (Alescio and Cassini, 1962; Warburton and Olver, 1997). Thyroid transcription factor-1 (TTF-1) expresses in the thyroid, forebrain, and lung epithelial cells. A large number of studies have shown that NKX2.1 is the earliest marker of the lung endoderm (Cardoso and Lu, 2006; Guazzi *et al*., 1990; Lazzaro *et al*., 1991). NKX2.1 is at the center of the pulmonary epithelial development regulatory network, and the appearance of $NKX2.1^+$ cells in the anterior foregut endoderm indicates the fate of pulmonary endoderm determination (El-Hashash, 2018). NKX2.1 also plays a very important role in further lung development. Studies have shown that NKX2.1 directly regulates the expression of lung proximal and distal related genes by binding to the gene promoter region, such as multiple functional genes of type II alveolar epithelial cells (AT2 cell) including surfactant protein A (*SPA*), surfactant protein B (*SPB*), surfactant protein C (*SPC*) and surfactant protein D (*SPD*), type I alveolar epithelial cells (AT1 cell) gene *PDPN* and club cell gene (Boggaram, 2009; Bohinski *et al*., 1994; Bruno *et al*., 1995). Complete deletion of NKX2.1 in mice kills them; it was found that the esophageal trachea was not separated and the lung development was stagnated, only bilateral main bronchi were developed and the distal lung parenchyma was completely absent (Kimura *et al*., 1999; Minoo *et al*.,

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1999). Wnt2/2b and beta-catenin signaling pathways are very important for early endodermal differentiation, and Wnt2/2b knockout in mice can lead to complete lung loss, and the initial NKX2.1$^+$ cell loss in the lung endoderm was detected (Goss *et al.*, 2009). However, conditional overexpression of beta-catenin will lead to ectopia proliferation of NKX2.1$^+$ cells in the esophageal and gastric epithelium (Warburton *et al.*, 2010b). The above results suggest that the regulation of the Wnt2/2b signal on lung development is strictly limited to the starting area of lung development in the anterior foregut endoderm. Fibroblast growth factor (FGF) protein family plays an important role in the process of cell proliferation, migration, and differentiation. Studies have shown that the function of FGF is highly consistent in the occurrence of respiratory organs from *Drosophila* to mammals (Volckaert and De Langhe, 2015). FGF-1, FGF-2, FGF-4, FGF-8, and FGF-10 have shown the ability to induce branch formation (limb bud formation) (Jin *et al.*, 2018; Su *et al.*, 2014; Yuan *et al.*, 2018). During the lung development of mice, FGF-10 was secreted by mesenchymal cells around the lungs, and the lung buds highly expressed FGF-2 and FGF-10 (Igarashi *et al.*, 1998; Ohuchi *et al.*, 1997). Complete deletion of FGF-10 in mice was fatal, and the lung was found to be completely absent (Min *et al.*, 1998); these results suggest that FGF-10 is essential for lung development. Keratinocyte growth factor (KGF, also known as FGF7) is highly expressed in lung interstitial cells in late lung development (Post *et al.*, 1996). FGF-7 has a regulatory effect on the proliferation and differentiation of pulmonary epithelial cells, but there is no obvious abnormality in the mice with *FGF-7* knockout (Guo *et al.*, 1996); it suggests that FGF-7 plays an auxiliary role in lung development.

During lung development, various components of the extracellular matrix (ECM), such as extracellular basement membrane, laminin (LNs), collagen, basement membrane chitosan (Perlecan), fibromodulin, and fibronectin provide organizational support and biological signal, regulating cell proliferation and differentiation (Chen *et al.*, 2018; Matter and Laurie, 1994).

AT1 and AT2 Cell Property

Lung distal progenitor cells expressed NKX2.1 and SOX9, and the distal progenitor cells (NKX2.1$^+$/SOX9$^+$) eventually differentiated into AT1 and AT2 cells (Kadzik and Morrisey, 2012). The results of mouse fetal lung single-cell sequencing showed that AT1 cells and AT2 cells were both derived from bipotent progenitor cells (BP cells) during lung development to the cystic stage, and then AT1 cells and AT2 cells were further amplified and matured at the alveolar stage (Desai *et al.*, 2014; Treutlein *et al.*, 2014). Single-cell sequencing also showed that mouse lung BP cells expressed of AT1 cells marker podoplanin (PDPN, also known as T1α) and AT2 cells marker SPC (Desai *et al.*, 2014; Treutlein *et al.*, 2014), as well as distal alveolar progenitor cells marker SOX9 (Rockich *et al.*, 2013). Mouse lung BP cells (SPC$^+$/PDPN$^+$/SOX9$^+$) can express more SPC, SPB and downregulate early AT1 marker PDPN to generate mature AT2 cells (SPC^{++}/SPB$^+$/PDPN$^-$), or express more mature AT1 markers and downregulate AT2 markers to generate mature AT1 (PDPN^{++}/AQP5$^+$/SOX9$^-$) (Chen *et al.*, 2018; Treutlein *et al.*, 2014) (Fig. 1(B)). hESCs-derived lung BP cells (SPC$^+$/PDPN$^+$/SOX9$^+$) were recently reported (Chen *et al.*, 2018), and more detailed studies are needed to investigate the cell property, such as lineage tracing and single-cell sequencing.

The alveoli are a continuous epithelial structure composed of AT1 and AT2 cells. AT1 cells account for about 8% of the total number of pulmonary parenchyma cells but constitute more than 90% of the alveolar surface area (Weibel, 2009). AT1 cell morphology is specific, flat, scaly, with multiple branch structures, can widely contact the basal and capillary endothelial cells to improve gas exchange efficiency (Weibel, 2009). AT1 cells have been considered as terminally differentiated cells that lack the ability to divide and change phenotypes (McElroy and Kasper, 2004). A large number of animal experiments have shown that SPC$^+$ AT2 cells can differentiate into AT1 cells to repair alveolar structures when alveolar cells are injured (Barkauskas *et al.*, 2013; Nabhan *et al.*, 2018) (Fig. 1(C)). A few results have shown that, under

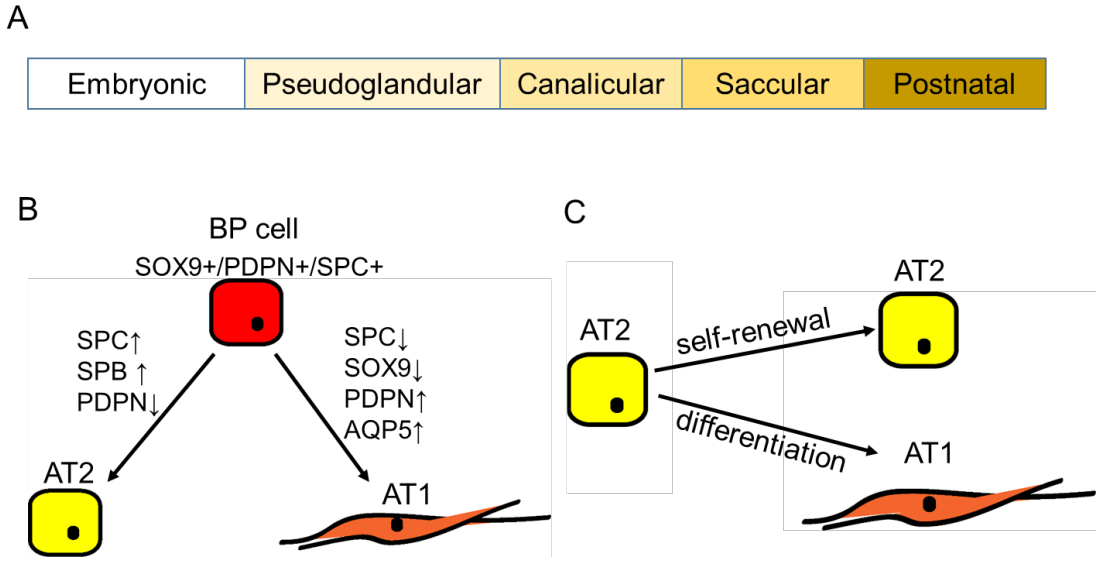

FIGURE 1. Lung development and AT1/AT2 cells property. (A) Continuous stages of human lung development. (B) AT1 and AT2 cells derived from BP cells (SOX9+/SPC+/PDPN+). (C) AT2 possessed self-renewal and differentiation capability.

certain circumstances, AT1 can transdifferentiate into AT2 cells (Flecknoe *et al*., 2002). Compared with a large number of studies on AT2 cells, an important reason why AT1 cells are less studied is the late identification of specific markers. Recent murine-based studies indicated that Hop Homeobox (HOPX) is the earliest detectable AT1 cell marker (Jain *et al*., 2015; Laresgoiti *et al*., 2016). The results of human fetal lung analysis also confirmed that HOPX could be detected in the distal bud area at 11 PCW (Nikolic *et al*., 2017). $HOPX^+$ AT1 cells possessed plasticity, could self-renew and differentiate into SPC^+ AT2 cells after partial pneumonectomy (Jain *et al*., 2015). A more recent study demonstrated that insulin-like growth factor-binding protein 2 (LGFBP2) as a genetic marker specifically expressed in postnatal AT1 cells, $HOPX^+$ $LGFBP2^-$ AT1 cells can transdifferentiate into AT2 cells, but $HOPX^+$ $LGFBP2^+$ cells are terminally differentiated AT1 cells that cannot proliferate and transdifferentiate into AT2 cells (Wang *et al*., 2018). It suggested that $HOPX^+$ AT1 cells population is composed of immature and mature AT1 cells. PDPN protein is an AT1 cell marker that appears later than HOPX (Laresgoiti *et al*., 2016), human fetal lung immunofluorescence staining also confirmed that PDPN expression could be detected in the distal alveolar area at 17 PCW (Nikolic *et al*., 2017). Although PDPN is also expressed in basal cells and lymphatic endothelial cells in the proximal trachea of the lung (Breiteneder-Geleff *et al*., 1999; Farr *et al*., 1992), but in the distal alveolar region, it had restricted expression in differentiating AT1 cells (Laresgoiti *et al*., 2016). Aquaporin 5 (AQP5) is considered as an AT1 cell marker that appears later than PDPN (Chen *et al*., 2018). Studies on human fetal lung have confirmed that PDPN+/AQP5+ AT1 cells can be detected in the terminal area of the pulmonary alveolar duct at 20 PCW, and staining results of the adult lung also confirmed that AT1 cells have high expression of AQP5 protein (Nikolic *et al*., 2017) (Tab. 1).

The results of single-cell sequencing in mice showed that the AQP5 was restricted expression in alveolar AT1 cells, while HOPX and PDPN were also expressed in alveolar progenitor cells (Desai *et al*., 2014). Moreover, AQP5 is a water channel protein that plays an important role in transporting water across the blood-gas barrier in the distal lung (Tao *et al*., 2016) and it is a functional marker of mature AT1 cells (Chen *et al*., 2018; Nikolic *et al*., 2017; Tao *et al*., 2016). The above-mentioned studies indicated that AQP5 protein was considered as a marker of mature AT1 cells (Fig. 2). A recent study showed that flattened AT1 cells undergoing terminal differentiation can be reprogrammed toward the airway fate, and proliferate, and that fully differentiated AT1 cells can retract their elaborate cellular extensions and proliferate (Yang *et al*., 2016). AT2 cells, in addition to the synthesis and secretion of alveolar surface-active substance and as stem cells that participate in alveolar damage repair function, have also the function to transfer sodium ions and alveolar fluid (Gonzalez *et al*., 2005; Mason, 2006).

TABLE 1

Overview of markers in distal progenitor and alveolar cells

Markers	BP	AT2	Immature AT1	Mature AT1
SOX9	+	+	+/−	−
PDPN	+	−	+	++
HOPX	+	−	+	+
LGFBP2	−	−	−	+
SPC	+	++	−	−
AQP5	−	−	−	++

++ means that marker higher expressed.
+/− means that marker expressed in basal level.

Future Directions

As more and more studies on lung development have been carried out, details on the properties of AT1 and AT2 cells get more clarity. Further studies based on single-cell sequencing and lineage tracing will reveal more biological features on AT1 and AT2 cells.

Conflicts of Interest

The authors declare no conflict of interest.

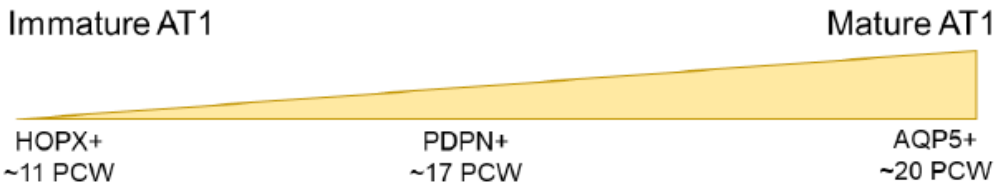

FIGURE 2. Markers expression of immature and mature AT1 cells.

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