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## SPOTLIGHT

# Modeling human lung development and disease using pluripotent stem cells

Hans-Willem Snoeck[1,2,3,*]

### ABSTRACT

Directed differentiation of human pluripotent stem cells (hPSCs) into mature cells, tissues and organs holds major promise for the development of novel approaches in regenerative medicine, and provides a unique tool for disease modeling and drug discovery. Sometimes underappreciated is the fact that directed differentiation of hPSCs also provides a unique model for human development, with a number of important advantages over model organisms. Here, I discuss the importance of using human stem cell models for understanding human lung development and disease.

### Introduction

The ability to generate tissues and organs from human pluripotent stem cells (hPSCs), in particular from human induced pluripotent stem cells (iPSCs), for tissue or organ replacement therapy is a very important, but in many cases distant, goal (Fox et al., 2014; Murry and Keller, 2008). In the shorter term, hPSC-derived differentiated cells are used for disease modeling and drug discovery (Lancaster and Knoblich, 2014; Robinton and Daley, 2012). However, an underappreciated rationale for directed differentiation of hPSCs is its use as a model for human development (Lancaster and Knoblich, 2014). Efforts to establish and validate directed differentiation of hPSCs as a model for human development will transform our understanding of human biology and disease. In this Spotlight article, I will illustrate these notions with directed differentiation of hPSCs into lung and airway tissue.

### Importance of modeling human development using hPSCs

Directed differentiation of hPSCs attempts to recapitulate development by sequentially applying carefully timed developmental signals to induce specification and maturation of hPSCs into a specific linage (Murry and Keller, 2008; Nostro and Keller, 2012). Because of the conservation of mechanisms involved in the establishment of the body plan and in organ domain specification, insights from mouse development are the scientific underpinning of this approach (Chambers et al., 2009; D'Amour et al., 2005; Gouon-Evans et al., 2006; Kubo et al., 2004; Nostro and Keller, 2012; Pagliuca et al., 2014; Spence et al., 2011; Yang et al., 2008). However, a common problem in this field is that full, terminal maturation of tissues appears difficult to achieve (Fox et al., 2014; Lancaster and Knoblich, 2014). The mechanisms involved in terminal differentiation and architectural maturation of organs and tissues are less well understood than those involved in early germ layer and organ domain specification. This problem is compounded by the fact that, in many organs, human and mouse organogenesis diverge more as development proceeds.

In addition to a 3000-fold difference in body mass, which profoundly affects metabolism, organ size, architecture and function, both species have evolved to adapt to very different habitats and positions in the food chain. The mouse model might therefore be less informative for human development at this stage.

Better insight into human development, gained from efforts at directed differentiation of hPSCs, will in turn improve differentiation strategies that might lead to applications in regenerative medicine. The ability to generate mature, fully differentiated cells will also allow disease modeling. Many human diseases have a developmental origin, either through acquired or inherited mutations, through intrauterine exposure to toxins or through deficiencies in essential nutrients or vitamins that affect development. Furthermore, many diseases are the result of the interaction between environmental exposure and genetic predisposition (which might have a developmental origin). Equally important, tissue-specific responses to disease or injury set in motion mechanisms aimed at regeneration or adaptation to chronic injury. These are often aberrant and pathological, and can co-opt pathways involved in development.

Directed differentiation of hPSCs might also shed light on the biology of human adult stem cells. Many tissues, such as skin (Blanpain and Fuchs, 2009), intestine (Clevers, 2013), muscle (Shadrach and Wagers, 2011), lung (Green et al., 2013; Rock and Hogan, 2011) and the hematopoietic system (Orkin and Zon, 2008), are endowed with stem cells that ensure repair from damage induced by wear and tear, cellular turnover and injury. In contrast to PSCs, adult stem cells are generally difficult to maintain, grow and expand *in vitro*. Owing to these challenges, their identity, functional characteristics and physical location are still debated in many organs, in particular in humans (Joseph and Morrison, 2005; Rando, 2006). The ability to generate adult stem cells in large quantities from hPSCs would therefore have major scientific and clinical implications.

### The lung: development and disease

The example of the lung makes a very strong case for the importance of using directed differentiation of hPSCs to understand human development and disease. An hPSC-derived model that faithfully recapitulates human development is a prerequisite for the development of hPSC-derived lung and airway cells for regenerative medicine. Indeed, non-malignant lung disease kills >100,000 people in the USA every year (Lewis et al., 2009). Transplantation is a therapeutic option, but is hampered by low availability of donor organs, and by severe surgical, medical and immunological complications (McCurry et al., 2009). Innovative approaches are therefore urgently needed.

Generating lung tissue from hPSCs is challenging, and has lagged behind efforts at directed differentiation into many other cell and tissues types. Recent work has shown, however, that anterior foregut endoderm (AFE; the structure from which the lung develops) can be generated from hPSCs (Green et al., 2011), and can subsequently be specified into developmental lung field progenitors, capable of

[1]Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY, USA. [2]Department of Medicine, Columbia University Medical Center, New York, NY, USA. [3]Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY, USA.

*Author for correspondence (hs2680@columbia.edu)

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further differentiation *in vivo* and *in vitro* (Huang et al., 2014). To model lung development, in particular terminal, distal lung development, three-dimensional (3D) approaches are required. Indeed, 3D organoid cultures are revolutionizing the study of human development (Lancaster and Knoblich, 2014). Such organoids have been generated from other organs, such as pituitary (Xia et al., 2013), liver (Takebe et al., 2013), kidney (Taguchi et al., 2014; Takasato et al., 2014), brain (Lancaster et al., 2013), eye (Nakano et al., 2012) and intestine (Spence et al., 2011), but this has yet to be achieved for the lung.

### Lung development

The respiratory system consists of a complex, branched system of progressively smaller airways that terminate in alveoli where gas exchange takes place (Weibel and Gomez, 1962). Lung and airways originate from buds that arise on the ventral aspect of the anterior foregut endoderm (AFE). These develop through a tightly regulated branching process, guided by surrounding mesoderm, into proximal airways and distal alveoli. Alveolar maturation occurs late in development and continues after birth, with the generation of alveolar epithelial type I (ATI), essential for gas exchange, and type II (ATII) cells, which produce surfactant, crucial for the maintenance of alveolar integrity (Herriges and Morrisey, 2014; Morrisey and Hogan, 2010; Whitsett et al., 2010).

The histology of lung and airways and the dynamics and timing of their development differ strikingly between human and mouse (Plopper and Hyde, 2008; Smith et al., 2010). A pseudostratified epithelium, containing basal cells, stem cells of the airway, submucosal glands and cartilage rings, is limited to the trachea and large lobar airways in the mouse (Morrisey and Hogan, 2010). This more complex epithelium extends to terminal bronchioles in the human. In contrast to the human airways, mucus-producing goblet cells are rare and secretory club cells (Clara cells) are abundant in the mouse. It is unclear to what extent environmental exposure plays a role in this difference. The acinus, the distal lung structure consisting of respiratory bronchioles, alveolar ducts and associated alveoli, is more complex in humans than in rodents. The mouse airways have approximately 12 branching generations, whereas the human airways have more than 20, suggesting a different pace of development relative to length of gestation (Plopper and Hyde, 2008).

Similarly, the pace of alveolar development and maturation differs between mouse and human (Plopper and Hyde, 2008; Smith et al., 2010) (Fig. 1). The first stage of development is the pseudoglandular stage, when branching morphogenesis takes place. During the subsequent canalicular stage, proximodistal differentiation of airway progenitors in the branching system becomes evident, with cells in the stalks expressing markers of bronchial epithelium. This stage is followed by the saccular stage, in which alveolar sacs, containing distinguishable ATI and ATII cells, form and surfactant secretion begins (Herriges and Morrisey, 2014; Morrisey and Hogan, 2010; Warburton et al., 2010). The saccular stage begins in late gestation, at E17.5, in the mouse, but relatively much earlier, at week 24, in humans (Smith et al., 2010). In the mouse, ATII and ATI cells probably develop from bipotential alveolar progenitors in the very tips of the branches, distal to the forming sacs (Desai et al., 2014; Treutlein et al., 2014). It is not known whether such bipotential progenitors exist in humans, or which signals determine the timing and direction of their lineage decisions. Furthermore, the mechanisms responsible for the initiation of sacculation and its developmental timing are unknown. Expansion of alveolar number by further differentiation of immature saccules, alveolar maturation and secondary septation continues postnatally. In fact, 85% of the so-called alveolar stage takes place postnatally. In humans, exponential growth takes place in the first two years, and a slower increase in alveolar number continues into puberty. In rodents, exponential growth occurs in the first 20 days of life, and stops more abruptly (Herring et al., 2014).

### Modelling human lung disease

The mouse has proved to be an imperfect model for several diseases affecting lung and airway, providing a strong rationale for developing and investigating models of human lung development. For example, mice with deletion of the *Cftr* gene, mutation in which causes cystic fibrosis in humans, lack signs of disease (Ratjen and Doring, 2003). The pathogenesis of one of the most frequent developmental abnormalities of the airway and gastrointestinal tract, tracheoesophageal fistula/esophageal atresia (TEF/EA), is unclear. Most commonly, children born with this very serious congenital malformation have a blind-ending proximal esophagus (esophageal atresia), whereas, more distally, trachea and distal esophagus are connected (tracheoesophageal fistula). Although several genetic mouse knockout models show features of TEF/EA, very few of those genes are mutated in humans with this congenital anomaly, and the etiology of most cases of TEF/EA in humans is not known (Bednarczyk et al., 2013).

Lung immaturity is the most threatening problem facing prematurely born infants, leading to bronchopulmonary dysplasia, which is characterized by decreased alveolarization and impaired secondary septation of alveoli (Smith et al., 2010). Given the very different architecture and developmental pace in the human compared with the mouse lung, understanding terminal human lung development is crucial for developing better therapeutic approaches. A subset of almost universally lethal pediatric lung diseases are due to surfactant deficiency, caused by mutations in genes encoding



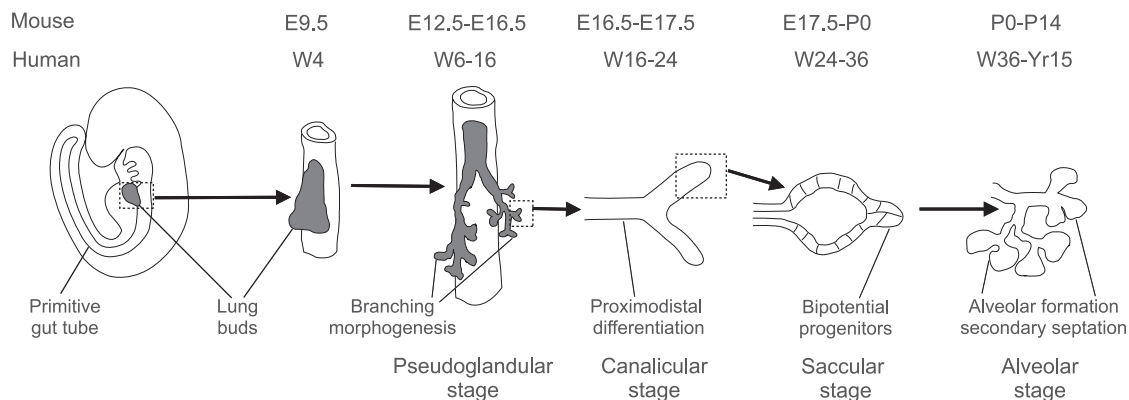| Mouse | E9.5 | E12.5-E16.5 | E16.5-E17.5 | E17.5-P0 | P0-P14 |
| Human | W4 | W6-16 | W16-24 | W24-36 | W36-Yr15 |

Fig. 1. Schematic representation of the stages of lung development and their approximate timing in human and mouse.

surfactant proteins (SP-C, SP-B) or factors required for surfactant trafficking (ABCA3). The clinical and pathological features of these diseases vary, however, and no therapy is available (Whitsett et al., 2010). Mouse models generated by deletion of the genes involved recapitulate the most severe forms of the disease and do not reproduce the clinical spectrum associated with the multitude of mutations observed in human populations (Ban et al., 2007; Clark et al., 1995; Fitzgerald et al., 2007; Glasser et al., 2001; Hammel et al., 2007). iPSC-derived models will therefore allow deeper insight into pathogenesis and might allow screening for drugs that could correct the phenotypes caused by at least some specific mutations. The etiology and pathogenesis of a number of severe congenital pediatric lung diseases that are not associated with surfactant deficiency – such as pulmonary acinar dysplasia (Gillespie et al., 2004; Rutledge and Jensen, 1986), congenital alveolar dysplasia (Mac, 1948) and neuroendocrine hyperplasia of infancy (Deterding et al., 2005) – is unknown, and mouse models are non-existent (Dishop, 2011). The availability of iPSC-based models of human lung development would shed light on the pathogenesis of pediatric congenital lung diseases, and might lead to novel therapeutic approaches.

Examining human lung development in hPSC-based models would also enhance our understanding of adult lung diseases. One of the most prevalent lung diseases, chronic obstructive lung disease (COPD), is characterized by small airway remodeling, including an increase in the proportion of goblet cells and mucus production (mucous cell metaplasia and hyperplasia). The underlying mechanism is unclear. During development, Notch signaling enhances the formation of secretory cells types in the mouse (Tsao et al., 2009). Similarly, sustained Notch signaling directs differentiation of basal cells, the stem cells of the large airway, towards a secretory fate (club and goblet cells) (Rock et al., 2011), and induces mucous metaplasia in human tracheal explants (Guseh et al., 2009). The same pathway therefore regulates normal development of mucous-producing cells and potentially regulates their overabundance in COPD in response to environmental noxious stimuli such as cigarette smoke. Understanding the development and homeostasis of goblet cells in a model that recapitulates the development of the human lung, in which goblet cells are more frequent than in mouse, might therefore provide crucial insights into COPD. COPD is a result of environmental exposure in genetically susceptible individuals (Holtzman et al., 2014). Although there is a clear role for the inflammation and the innate immune system (Holtzman et al., 2014), it is possible that the predisposition to airway remodeling in susceptible individuals has at least in part a developmental origin that could be revealed through studies of patient-specific human development. Similarly, predisposition to bronchial hyperreactivity in asthma might find its origin in development (Sharma et al., 2014).

Another highly prevalent but intractable lung disease is idiopathic pulmonary fibrosis (IPF), which kills approximately 40,000 Americans each year (Noble et al., 2012; Ryu et al., 2014). Although several genetic polymorphisms and mutations predispose to IPF, its pathogenesis is unclear, and there is no good mouse model. One of the predisposing mutations causes aberrant processing of SP-C, resulting in an unfolded protein response. As SP-C is specifically expressed in ATII cells, these findings suggest a central role for these cells in pathogenesis. ATII cells are now accepted as at least one of the facultative stem cell populations involved in distal lung regeneration (Barkauskas et al., 2013; Desai et al., 2014). It is hypothesized that aberrant repair by ATII cells leads to fibrosis, although the origin of the mesenchymal cells, and how the fibrotic process is initiated and sustained, is not known. Other mutations or polymorphisms associated with IPF affect genes that are not specifically expressed in ATII cells, such as telomerase (Armanios et al., 2007), or are not expressed in ATII cells, such as MUC5B (Seibold et al., 2011). How these mutations relate to disease is largely unclear. ATII cells can currently not be isolated *ex vivo* and maintained *in vitro*. Furthermore, ATII cells isolated from patients after diagnosis (typically postmortem or at the stage of terminal respiratory failure requiring transplantation) might not be informative for disease pathogenesis and predisposition, as many observed changes in expression patterns might be secondary to the terminal fibrotic disease process. However, human ATII cells can now be generated from hPSCs with high efficiency (Huang et al., 2014), providing a model to study their biology and their role in IPF. IPF is also a disease in which genetic predisposition and environmental exposure determine disease penetrance (Noble et al., 2012; Ryu et al., 2014). An open question is whether individuals susceptible to IPF in fact do have subtle developmental lung abnormalities that predispose to aberrant, ATII-mediated responses to environmental injury. Studies on iPSC-derived ATII cells from patients and their unaffected relatives, as well as unrelated controls, could shed light on this question.

Finally, the lung is endowed with enormous regenerative capacity after injury. Studies in the mouse indicate that the nature of the recruited stem cell populations is regionally distinct and dependent on the type of injury (Chapman et al., 2011; Desai et al., 2014; Kumar et al., 2011; Rock and Hogan, 2011). Virtually nothing is known on the location, phenotype and developmental origin of stem cell populations in the human lung, in particular in the distal lung. hPSC-derived models of human lung development might provide innovative strategies to identify postnatal stem cell populations, and to gain insight into their developmental origin and regulation.

## Concluding remarks

As illustrated above using the example of the lung, there is an urgent need to better understand human organogenesis, as this is in many cases a prerequisite for developing models and potential therapies for human disease. Directed differentiation of hPSCs and generation of organoids provides a tool for such studies, and this might provide unparalleled insight into human development, pathogenesis and disease predisposition.

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