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Human airway organoid engineering as a step toward lung regeneration and disease modeling

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ABSTRACT

Organoids represent both a potentially powerful tool for the study cell-cell interactions within tissue-like environments, and a platform for tissue regenerative approaches. The development of lung tissue-like organoids from human adult-derived cells has not previously been reported. Here we combined human adult primary bronchial epithelial cells, lung fibroblasts, and lung microvascular endothelial cells in supportive 3D culture conditions to generate airway organoids. We demonstrate that randomly-seeded mixed cell populations undergo rapid condensation and self-organization into discrete epithelial and endothelial structures that are mechanically robust and stable during long term culture. After condensation airway organoids generate invasive multicellular tubular structures that recapitulate limited aspects of branching morphogenesis, and require actomyosin-mediated force generation and YAP/TAZ activation. Despite the proximal source of primary epithelium used in the airway organoids, discrete areas of both proximal and distal epithelial markers were observed over time in culture, demonstrating remarkable epithelial plasticity within the context of organoid cultures. Airway organoids also exhibited complex multicellular responses to a prototypical fibrogenic stimulus (TGF-β1) in culture, and limited capacity to undergo continued maturation and engraftment after ectopic implantation under the murine kidney capsule. These results demonstrate that the airway organoid system developed here represents a novel tool for the study of disease-relevant cell-cell interactions, and establishes this platform as a first step toward cell-based therapy for chronic lung diseases based on de novo engineering of implantable airway tissues.

1. Introduction

Respiratory diseases such as chronic obstructive pulmonary disease and pulmonary fibrosis represent a large and growing public health burden [1,2], are associated with substantial morbidity and mortality, and currently lack curative therapies. At their end-stage, such diseases require lung transplantation for therapy, but the supply of donor organs is extremely limited, and lung transplant outcomes remain suboptimal [3]. Regenerative approaches offer potential long-term hope for addressing both the epidemic of chronic lung diseases, and the shortage of donor organs, but critical hurdles remain to be overcome [4]. While recent studies have made great progress delineating the mechanisms of lung development and developing methods to drive iPS cells toward mature lung lineages [5–7], relatively less progress has been made in designing strategies by which these advances might be translated into tissue repair, and ultimately advanced toward human studies. Current approaches to engraft dissociated cells in the lung show promise, but have thus far been limited to the setting of severe infections [8] or radiation-induced preconditioning [9]. A major alternative emphasis has been on the generation of decellularized and recellularized lung scaffolds as an engineered organ replacement [10–13]. Relatively less attention has been devoted to the de novo generation of complex three-dimensional lung-like tissues in culture suitable for eventual translational applications. A potential additional benefit from developing such complex engineered lung tissues is for disease modeling. Chronic lung diseases are distinguished by specific tissue remodeling processes and complex cell-cell interactions that are not easily recapitulated in typical cell culture systems. Therefore, we sought to develop an airway organoid culture system combining multiple lung cell types as both a step toward eventual regenerative approaches, and as a system to study disease-relevant cell-cell interactions and complex

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tissue remodeling processes.

To generate highly organized 3D tissues that mimic organ structure and function, tissue engineers have attempted to recapitulate the in vivo organogenesis process by manipulating critical aspects of the cell culture environment. During embryonic development, the lungs and other internal organs first emerge as organ buds composed of epithelial and mesenchymal progenitors. Through repeated rounds of outgrowth and branching primitive organ buds grow into mature organs [14]. The reciprocal epithelial-mesenchymal interactions critical to organogenesis during embryonic development can be recapitulated in three dimensional co-culture systems to guide formation of similar tissue-like structures in vitro [15]. Recently, complex structures termed organoids [16] have been generated for brain [17], liver [18], pancreas, and lung [19] using combinations of induced pluripotent stem cells, inductive soluble factors, and supportive three dimensional culture conditions. Alternatively, resident progenitor cells from adult tissues can be cultured in supportive 3D systems, and can also generate organoids. Typical examples include LGR5+ cells from intestine and liver [20], and in the field of lung biology, the generation of tracheospheres [21] and alveospheres [22,23] from airway and alveolar epithelial progenitors. While organoids have shown promise in transplantation models in the colon [24] and liver [25], similar advances have not been reported using adult-derived lung progenitors. Similarly, although organoids have potential for disease modeling and drug screening, tractable human lung cell-based organoid systems have not been reported.

Here we combined adult human primary bronchial epithelial cells, lung fibroblasts, and lung microvascular endothelial cells in 3D culture conditions to generate airway organoids. By combining epithelial differentiation conditions with a multicellular aggregation culture system, we generated self-assembling bioengineered airway organoids that are amenable to ectopic transplantation and study of cell-cell interactions crucial to tissue biology. This system represents a novel tool for studying disease-relevant cellular and molecular function, and an important step toward cell-based therapy for chronic lung diseases based on de novo engineered airway tissues.

2. Material and methods

2.1. 2D cell culture and labeling

Human bronchial epithelial cells (NHBE, 8 cell lines used) were purchased from Lonza and cultured in bronchial epithelial growth medium (BEGM, Lonza) with 1% Antibiotic-Antimycotic. Human microvascular lung endothelial cells (HMVEC-L, 3 cell lines used) were also purchased from Lonza and cultured in Endothelial Cell Growth Medium (EBM-2MV, Lonza) with 1% Antibiotic-Antimycotic. Human lung fibroblasts (generously provided by Carol Feghali-Bostwick) previously isolated by explant culture from donor lungs rejected for transplantation under a protocol approved by the University of Pittsburgh Institutional Review Board, were culture in DMEM with 10% FBS, 1% Antibiotic-Antimycotic. The cells were regularly maintained in humidified 5% CO2 at 37 °C. For cell labeling, cells were incubated with pre-warmed CellTracker™ Working Solution (1 μM from 1000X DMSO dissolved stock solution) for 30 min.

2.2. 3D cell culture and generation of human airway organoids

Culture plates were coated with 40% Matrigel (Corning) combined with PneumaCult-ALI Maintenance Medium (Stemcell Technologies). The former is an extracellular matrix preparation derived from a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells, composed of approximately 60% laminin, 30% collagen IV, and 8% entactin. Entactin is a bridging molecule that interacts with laminin and collagen IV, and contributes to structural organization. Although Matrigel has inherent long term limitations for clinical use, it is a valuable proof of concept matrix system for supporting organoid morphogenesis [26]. PneumaCult-ALI is a media formulation optimized to induce primary human bronchial epithelial cell mucociliary differentiation under air-liquid interface (ALI) culture conditions. After 45 min incubation at 37 °C for gelation of the thick Matrigel layer, a single cell suspension including NHBE, HMVEC-L and HLF cells was combined with 5% Matrigel and PneumaCult- ALI Maintenance Medium, and was seeded on top of the Matrigel layer. The ratio of each type of cell was human NHBE:HMVEC-L:HLF = 10:7:2 following a previously described approach for generation of liver organoids [18]. The cells were fed with 5% Matrigel in PneumaCult-ALI Maintenance Medium every other day and observed by light microscopy. Cells labeled with Fluorescent CellTracker™ were observed in a Cytation 5 Cell Imaging Multi-Mode Reader for 24 h time-lapse fluorescence microscopy to visualize organoid compaction and formation.

2.3. Organoid actomyosin and YAP analysis

Day 2 organoids were used for treatment. ON-TARGETplus Human YAP1 (10413) siRNA SMARTpool (30 pmol, Dharmacon) in Opti-Mem medium (Life Technologies) was transfected using Lipofectamine RNAi Max (Life Technologies) followed by incubation for 24 h at 37 °C, with media than changed to the normal differentiation medium for another 48 h. Non-muscle myosin inhibitor blebbistatin (10 μmol, Sigma, B0560), F-actin inhibitor cytochalasin D (10 μmol, Sigma, C8273) and Rho kinase inhibitor Y-27632 (100 μmol, Stemcell Technologies, 72304) were used to treat day 2 organoids for 72 h. Photos were taking by Nikon TMS Microscope or Cytation 5 Cell Imaging system. Buds or invasive tubular structures were counted manually and average diameters of organoids were counted in 3 organoids per group using ImageJ.

2.4. Atomic force microscopy

Organoids were embedded in O.C.T (Optimal Cutting Temperature, n°4583) and stored at −80 °C. 10 μm thickness organoid slices were cut by cryosection (Leica) at −21 °C and mounted on poly-L-lysine coated glass slides. To avoid drying, tissue slices were maintained in PBS. Measurements were performed using a BioScope Catalyst AFM (Bruker) mounted on an inverted microscope equipped with epifluorescence (Olympus) using a spherical tip (Novascan) with a radius of 2.5 μm and a spring constant of ~98 pN/nm. Force curves were acquired with MIRO 2.0 (NanoScope 9.1, Bruker) at room temperature in PBS. The indentation was estimated at ~250 nm for an applied force of ~24 nN. The preparation of organoid slice sample and the AFM measurements were performed on the same day. Force curves were analyzed by NanoScope Analysis (Bruker). The extend curve was fitted to determine the Young's modulus using the Hertz model assuming Poisson's ratio of 0.4 [27].

2.5. Gene expression analysis (RNA isolation, cDNA synthesis, RT-PCR)

Total RNA was isolated with RNeasy Plus Mini kit and cDNA was synthesized with SuperScript™ IV Reverse Transcriptase. Gene expression levels were quantified by qRT-PCR on the Lightcycler 96 Real-Time PCR System (Roche) according to the manufacturer's instruction. qRT-PCR was performed by incubating at 95 °C for 10 min and then cycling 40 times at 95 °C for 10 s, 60 °C for 10s,