Endobronchial Ultrasound-Guided Pulmonary Arterial Injection of Airway Organoids Enables Peripheral Lung Engraftment in a Porcine Model

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Abstract

Lung transplantation remains the only curative therapy for end-stage lung diseases; however, donor shortages necessitate alternative regenerative strategies. Stem cell–derived organoids offer a potential therapeutic approach, but efficient delivery to the peripheral lung parenchyma has not been established. We investigated whether endobronchial ultrasound (EBUS)-guided pulmonary arterial (PA) injection could serve as an effective method for organoid transplantation in pigs. Yorkshire pig airway stem cells were established by conditional reprogramming, genetically labeled with Venus-Akaluc, and cultured to form airway organoids. In a 24-hour survival model (n=3), allogeneic organoids were transplanted into the right lung via PA injection or into the left lung via bronchial injection for comparison. Bioluminescence imaging revealed significantly stronger signal in the PA-injected lungs, with organoids distributed to peripheral parenchymal regions, whereas bronchially delivered organoids remained localized near the central airways. In a 7-day survival model (n=3), autologous organoids were transplanted following bleomycin-induced lung injury. IVIS imaging and histological analyses revealed that organoid-derived cells extravasated from the pulmonary vessels and then engrafted into the alveolar epithelium, differentiating into podoplanin-positive type I and surfactant protein C-positive type II alveolar epithelial cells. These findings suggest that EBUS-guided PA injection represents a promising strategy for airway organoid delivery to peripheral lung parenchyma and may provide a foundation for future regenerative therapies in end-stage lung diseases.

Introduction

Lung transplantation is the only definitive therapy for many forms of end-stage lung diseases such as chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF)[1]. However, there are not enough suitable donor lungs to meet the needs of all patients with end-stage lung disease, which results in the eventual death of many patients waiting for transplants[1,2]. In order to help these patients, alternative therapeutic approaches including, stem cell therapies, are highly needed.

Stem cell therapy has the potential to repair the impaired lung function through lung regeneration[3]. Stem cell therapy clinical trials have been conducted using mesenchymal stem cells[4] and type II alveolar cells[5]. However, these clinical trials demonstrated only limited benefits. In 2015, Zuo et al. reported P63 + KRT5+ distal airway stem cells are essential for lung regeneration and these cells can differentiate into type I, II alveolar cells, as well as bronchiolar cells in mouse lung[6]. Since then, efficient culture protocols of P63 + KRT5+ airway stem cells in human and mouse have been reported[7].

Organoid culture is a recently developed culture method, and organoids are organ “like” structures that are derived from stem cells. Organoids can be transplanted for a regenerative purpose[8]. For example, cultured colon organoids were transplanted into the damaged colon epithelium in mice, and these organoids rescued the damaged colon epithelium[9]. Organoid transplantation to treat inflammatory bowel disease is currently under evaluation in a clinical trial in Japan (JRCTb032190207). As for lung,

organoids containing lung progenitors were reported to be passageable maintaining their differentiation capacity[10–13]. iPS cell derived lung organoids transplantation into a damaged mouse lung has been reported recently[14]. In this article, organoids were transplanted intrabronchially, and could differentiate into lung epithelial cells, however, the engraftment was mainly observed around the central bronchial regions, and the overall efficiency appeared limited. Subsequently, we considered improving the organoid transplantation method into the lung for an accelerated organoid therapy for lung regeneration. We hypothesized that pulmonary arterial (PA) injection of organoids might be a better way to inject organoids rather than bronchial injection because there is a blood stream going towards the peripheral lung parenchyma, and the transplanted organoids would always have access to nutrient rich blood in a pulmonary artery. Additionally, a PA injection may also prevent loss of transplanted organoids caused by coughing, mucociliary clearance, or sputum expectoration, which are inherent limitations of bronchial injection.

Endobronchial ultrasound (EBUS)-guided transbronchial needle injection (TBNI) is a novel technique for treating peribronchial targets[15]. Our team has also recently developed a treatment method for pulmonary embolism by implementing EBUS-TBNI[15–17]. For this, tissue plasminogen activator (t-PA) was directly injected into a pulmonary artery clot under EBUS guidance.

This led us to consider EBUS-TBNI as a good option to deliver organoids into the pulmonary artery. The purpose of this study is to investigate whether PA injection of organoids by EBUS-TBNI is an effective method to deliver organoids to lung parenchyma for the purpose of lung regeneration.

Materials and methods

Animals

Total of six (n=6) Yorkshire pigs weighing 20-30kg were used in this study. All animal experiments were approved by the University Health Network Animal Care Committee (AUP6763).

Anesthesia

The pigs were premedicated with intramuscular ketamine (20 mg/kg; Bimeda Inc, USA), midazolam (0.3 mg/kg; Fresenius Kabi, Germany), and atropine (0.04 mg/kg; CDMV Inc, Canada), followed by anesthetic induction with isoflurane (Fresenius Kabi, Germany). The pigs were positioned supine, intubated (internal diameter 7-8 mm; Mallinckrodt Pharmaceuticals, Ireland), and ventilated. Anesthesia was maintained with 2-3% isoflurane in oxygen. Vital parameters including heart rate, oxygen saturation, and end-tidal CO_2 were monitored throughout the procedure.

Establishment, culture of airway organoid

Pig airway stem cells were established from biopsied pig bronchial tissue using the conditional reprogramming (CR) method, as previously described[18,19]. Briefly, bronchial tissue specimens were

minced and enzymatically dissociated. The isolated epithelial cells were cultured under CR conditions with irradiated mouse 3T3-J2 feeder fibroblasts (ATCC, USA) in F-medium consisting of DMEM/Ham's F-12 (Thermo Fisher Scientific, USA) supplemented with 5% fetal bovine serum (FBS, Gibco, USA), insulin (5 µg/ml, Sigma-Aldrich, USA/Germany), hydrocortisone (0.4 µg/ml, Sigma-Aldrich, USA/Germany), cholera toxin (8.4 ng/ml, Sigma-Aldrich, USA/Germany), adenine (24 µg/ml, Sigma-Aldrich, USA/Germany), and the ROCK inhibitor Y-27632 (10 µM, Selleck Chemicals, USA). This culture system enabled efficient proliferation while maintaining stem/progenitor-like characteristics.

For airway organoid generation, 1.0 × 10^5 expanded pig airway stem cells were resuspended in 50 µl of growth factor–reduced Matrigel (Corning, USA) and seeded as domes in 24-well plates. After gelation at 37 °C, 500 µl of organoid culture medium was added to each well. The medium contained Advanced DMEM/F12 (Thermo Fisher Scientific, USA) supplemented with B27 (1X, Gibco, USA), GlutaMax (2mM, Gibco, USA), Antibiotic-Antimycotic (100 U/ml, Gibco, USA) HEPES (10 mM, Gibco, USA), N-Acetyl-L-cysteine (1.25 mM, Sigma-Aldrich, USA), EGF (50 ng/ml, PeproTech), Noggin (100 ng/ml, PeproTech, USA), FGF4 (100 ng/ml, PeproTech, USA), FGF7 (25 ng/ml, PeproTech, USA), A83-01 (0.5 µM, Tocris, UK), SAG (100nM, Enzo, USA) CHIR99021 (3 µM, Tocris, UK), and Y-27632 (10 µM, Sellek Chemicals, USA) as described before[20]. Under these conditions, spherical cystic organoids were typically observed within 7 days and were used for transplantation on day 10 of culture. Organoid size was assessed from bright-field microscopy images. For each experiment, 30 well-focused organoids were randomly selected, and their diameters were measured using ImageJ (NIH, USA).

Genetic Labeling of Airway Stem Cells

To facilitate *in vivo* tracking, airway stem cells were transduced with a lentiviral vector generated from pLenti-PGK-Venus-Akaluc (neo) obtained from Addgene (plasmid #124701). After transduction, cells were cultured with 100 µg/mL of G418 (Geneticin; Thermo Fisher Scientific, USA) to enrich for stable Venus-Akaluc–expressing populations. Stable Venus-Akaluc expression was verified by fluorescence microscopy and bioluminescence assays prior to organoid generation.

Organoid transplantation

In the 24-hour survival model, allogeneic airway organoid transplantation was performed to compare two different delivery routes within the same animal. Immediately prior to organoid administration, recipient pigs received intravenous methylprednisolone (500 mg; Pfizer, USA) to induce systemic immunosuppression.

Prior to transplantation, airway organoids were harvested from Matrigel by dissolving the matrix using Cell Recovery Solution (Corning, USA) on ice for 1 hour. The organoids were resuspended in 5ml sterile phosphate-buffered saline (PBS, Gibco, USA) and injected at a final dose of 5.0 × 10^5 cells per kilogram body weight. The number of transplanted cells was estimated by dissociating organoids cultured in parallel wells and counting the constituent cells using a hemocytometer. This value was used to calculate the approximate total number of cells administered for transplantation.

EBUS and EBUS-TBNI were performed with a convex probe EBUS bronchoscope (BF-UC180F; Olympus Corporation) with a dedicated ultrasound processor (EU-ME1; Olympus Corporation). A 22gauge ViziShot 2 EBUS-TBNA needle (NAU401SX-4021-A, Olympus Corporation) was used for organoid injection. Correct needle placement was further confirmed by fluoroscopy prior to injection.

For PA injection, the suspension was injected into the right basal pulmonary artery using an EBUS at the level of the third dorsal (D3) bronchus, at a controlled rate of 5 ml/min under real-time EBUS guidance. In parallel, an equivalent dose and volume of organoid suspension were injected into the left basal bronchial lumen using a bronchoscope at the level of the D3 bronchus. These procedures enabled precise delivery of airway organoids into either the pulmonary artery or the bronchial lumen for comparative evaluation. Animals were maintained for 24 hours before evaluation of organoid engraftment and distribution.

In the 7-day survival model, autologous airway organoid transplantation was performed via the right pulmonary artery under EBUS guidance. To prepare autologous donor cells, bronchial tissue surrounding the fourth dorsal (D4) bronchus was biopsied 30 days prior to transplantation (day -30), and airway stem cells were established using the CR method. On day -23, the Venus-Akaluc reporter gene was transduced into the airway stem cells, and transduced cells were expanded for organoid generation. On day -7, pulmonary injury was induced by instilling bleomycin (1 U/kg; Fresenius Kabi, Germany) into the basal segments of both lower lobes.

The immunosuppressive protocol was based on previously reported regimens[21]. Recipient pigs received daily cyclosporin A (10–16 mg/kg/day; Novartis, Switzerland) in combination with tapering doses of methylprednisolone (125-250 mg/day; Pfizer, USA), starting three days prior to transplantation. On the day of transplantation, additional immunosuppression of intravenous abatacept (12.5 mg; Bristol-Myers Squibb, USA) administered immediately prior to organoid delivery. Blood concentrations of cyclosporin A were monitored twice weekly to ensure adequate immunosuppression levels.

For transplantation, organoids were harvested from Matrigel by dissolving the matrix using Cell Recovery Solution (Corning, USA) on ice for 1 hour, resuspended in 5 ml of sterile PBS, and injected into the right basal pulmonary artery at the level of the D4 bronchus using an EBUS bronchoscope at a final dose of 1.0 × 10^6 cells per kilogram body weight. Animals were maintained for 7 days before euthanasia and subsequent evaluation of organoid engraftment and distribution was performed.

Ex vivo bioluminescence imaging analysis

For *ex vivo* bioluminescence imaging of both lung tissues and cultured organoids, TokeOni (Tocris Bioscience, UK) was used as the substrate. For organoids cultured *in vitro*, TokeOni was added directly to the culture medium to achieve a final concentration of 10 μM, followed by incubation for 15 minutes at room temperature prior to imaging.

For lung tissue analysis, resected lungs were immersed in a container filled with PBS, and TokeOni was added to achieve a final concentration of 10 μM. After 15 minutes of incubation at room temperature, bioluminescence signals were acquired using the Xenogen IVIS Spectrum system (PerkinElmer, USA) under standardized exposure settings. Quantitative analysis of photon flux was performed by defining regions of interest (ROI) using Living Image software (PerkinElmer, USA).

Histological analysis, immunohistochemistry, and immunofluorescence

Airway stem cells, airway organoids and resected lung tissues containing transplanted airway organoids were processed to evaluate histopathological findings.

Airway stem cells cultured on 24-well plates were fixed in 4% paraformaldehyde and subjected to immunofluorescence staining. Basal and progenitor marker expression was confirmed using a rabbit monoclonal anti-P63 antibody (Abcam, UK, ab124762; 1:200), a mouse monoclonal anti-KRT5 antibody (Biorbyt, UK, orb256030; 1:200), and a rabbit polyclonal anti-SOX9 antibody (Millipore, USS, AB5535; 1:200). Alexa Fluor 488- or 555-conjugated secondary antibodies (Invitrogen, USA; 1:100) were used, and nuclei were counterstained with DAPI (Bio-RAD). Fluorescence images were acquired using a Nikon fluorescence microscope (Nikon, Japan).

Airway organoids were retrieved from Matrigel, washed with PBS, embedded in Histogel (Thermo Fisher Scientific, USA), fixed in 10% neutral-buffered formalin, and paraffin-embedded. Lung tissues were processed in the same manner, and 4-μm sections were prepared. Regions corresponding to bioluminescence signals identified by IVIS analysis were examined in detail. Hematoxylin and Eosin (H&E) staining was performed for morphological evaluation. To identify Venus-Akaluc–labeled organoids in both lung tissue and airway organoid sections, GFP detection was performed by Immunohistochemistry (IHC/DAB method). Sections were incubated with a rabbitt polyclonal anti-GFP antibody (Abcam, UK, ab290; 1:1000), followed by an HRP-conjugated secondary antibody and visualization with 3,3'-diaminobenzidine (DAB; Vector Laboratories, USA).

For lineage analysis, double immunofluorescence staining was performed using the same anti-GFP antibody in combination with either a mouse monoclonal anti-podoplanin antibody (Santa Cruz, SC-59347; 1:50) to identify alveolar type I cells, or a mouse monoclonal anti-surfactant protein C (SP-C) antibody (Santa Cruz, SC-518029; 1:50) to identify alveolar type II cells. Fluorescence images were acquired using a Nikon fluorescence microscope, and co-localization was assessed in merged channels.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9 software (San Diego, CA, USA). Data are presented as mean ± standard deviation (SD). Comparisons between groups were conducted using two-way ANOVA. A p-value of <0.05 was considered statistically significant.

Results

Preparation of organoids for transplantation

Yorkshire pigs (20-30 kg) were used in this study. Airway stem cells were established from isolated bronchial cells from a donor Yorkshire pig bronchus by the CR method, and expressed the basal cell markers P63 and KRT5, as well as the progenitor marker SOX9, as demonstrated by immunofluorescence staining (Figure 1A-C, S1A). These cells were successfully transduced with the Venus-Akaluc reporter gene via lentiviral infection, and robust reporter expression was confirmed by fluorescence microscopy (Figure S1B). When cultured under organoid-forming conditions, the transduced airway stem cells generated spherical organoids within 7 days and were used for transplantation on day 10 of culture with a mean diameter of 78.8 μm (Figure 1D, E). Venus-Akaluc expression was stably maintained in the organoids, as verified by fluorescence microscopy and further confirmed by bioluminescence imaging using the Xenogen IVIS Spectrum system (Figure 1F, S1C). Venus-Akaluc expression was also confirmed immunohistochemically using an anti-GFP antibody (Figure 1G).

Comparison between Pulmonary arterial injection and bronchial injection in 24 hours after transplantation.

Venus-Akaluc transduced airway organoids (5.0 × 10^5 cells/kg) were injected into the right basal pulmonary artery at the level of the third dorsal (D3) bronchus under real-time EBUS guidance (Figure 2A, B; Movie 1). Subsequently, an equivalent dose was delivered into the left basal bronchial lumen at the D3 bronchus (Figure 2A, C; Movie 2). Organoid delivery was successful in all three animals without complications. Twenty-four hours after transplantation, the animal was sacrificed. The bilateral lower lobes were resected, divided axially into six sections, and incubated with the substrate Toke-Oni (10 μM) (Figure 2D). Bioluminescence signals were measured using the Xenogen IVIS Spectrum system 15 minutes after substrate exposure. Quantitative analysis revealed that the overall signal intensity was significantly higher in the right lower lobe (PA injection) compared with the left lower lobe (bronchial injection) ($p < 0.01$, two-way ANOVA) (Figure 2E, S2A). Notably, bronchially injected organoids remained localized around the central airways, whereas PA injection enabled efficient distribution to the peripheral lung parenchyma (Figure 2E). Histological examination further demonstrated that most transplanted organoids were retained within pulmonary vessels, although some cells were observed migrating beyond the vascular boundary (Figure S2B).

In summary, PA injection via EBUS achieved more efficient and widespread delivery of airway organoids to the peripheral lung parenchyma than conventional bronchial injection.

Confirmation of the extravasation of organoids transplanted into the pulmonary artery and their differentiation into alveolar epithelial cells.

Next, we investigated whether transplanted organoids extravasate from pulmonary vessels and engraft in the alveolar epithelium 7 days after PA injection. For this purpose, autologous transplantation was performed (Figure 3A, B). Airway stem cells were established from bronchial tissue around D4 bronchus 30 days prior to transplantation, and Venus-Akaluc was transduced 23 days prior to transplantation. To induce lung injury and assess regenerative potential, bleomycin (1 U/kg) was administered 7 days prior to transplantation. An estimated 1.0 × 10^6 cells were transplanted into the right lung via PA injection at the level of the D4 bronchus under EBUS guidance, while the left lung served as the control (non-transplantation).

Because Venus-Akaluc transduction might elicit an immune response even in the autologous setting, immunosuppression was initiated with cyclosporin A (10-16 mg/kg/day), methylprednisolone (125-250 mg/day), and abatacept (12.5 mg/kg). Computed Tomography (CT) imaging was performed 7 days before, on the day of, and 7 days after transplantation. Pneumonia was observed in both lungs immediately after transplantation and persisted for 7 days, with no clear differences between the transplanted and control lungs (Figure S3A).

Seven days after transplantation, *ex vivo* bioluminescence imaging demonstrated detectable signals in the right lung following PA injection (Figure 3C, S3B). It was challenging to maintain stable blood levels of the immunosuppressive agents, which may have negatively influenced the engraftment efficiency (Figure S3C). Histological analysis revealed GFP-positive organoid-derived cells that had exited the pulmonary vasculature and engrafted within the alveolar epithelium (Figure 3D). Double immunofluorescence staining confirmed that a subset of GFP-positive cells expressed podoplanin, consistent with differentiation into type I alveolar epithelial cells (Figure 3E). Additional double staining for GFP and SP-C revealed differentiation into type II alveolar epithelial cells (Figure 3E).

In summary, autologous organoid transplantation via the pulmonary artery enabled limited but detectable engraftment of airway organoids in alveolar epithelial regions, with evidence of differentiation toward both type I and type II alveolar lineages.

Discussion

In this study, we demonstrated that airway organoids can be effectively delivered to the peripheral lung parenchyma through EBUS-guided PA injection in a porcine model. Traditional intrabronchial transplantation methods have been hindered by poor engraftment, with transplanted cells largely remaining within central bronchi. Our findings show that PA injection enables transplanted organoids to reach the peripheral parenchyma through the bloodstream, where they are exposed to a nutrient-rich microenvironment that may enhance engraftment potential.

In the 24-hour survival model, PA injection achieved stronger bioluminescence signals and broader distribution compared with bronchial instillation, underscoring the advantage of vascular delivery. In the 7-day autologous transplantation model, we further confirmed that airway organoid–derived cells could extravasate from pulmonary vessels, integrate into alveolar structures, and differentiate into both type I

and type II alveolar epithelial cells. These lineages are indispensable for alveolar repair, as type I cells cover the gas-exchange surface while type II cells contribute to epithelial regeneration and surfactant production [4–6]. However, the extent of engraftment after 7 days was limited, and only a small fraction of transplanted organoids persisted in the alveolar regions. This modest engraftment represents an important limitation of our study. Notably, despite the autologous transplantation setting, the lentiviral introduction of the Venus-Akaluc reporter may have increased the immunogenicity of the transplanted cells, potentially contributing to reduced engraftment efficiency[22].

Our approach builds on prior studies of organoid transplantation, which have primarily relied on intrabronchial instillation or surgical implantation [6,7]. To our knowledge, this is the first report utilizing a clinically available EBUS-TBNI technique for organoid delivery into the pulmonary artery [8–10]. This approach enables efficient delivery of a large number of organoids to a targeted local region. Because EBUS is already widely adopted in pulmonary medicine for diagnostic and interventional procedures, this strategy has high translational potential.

There is also a potential concern that puncturing the pulmonary artery under EBUS guidance could lead to hemorrhagic complications. Nevertheless, existing studies, including our group's preclinical investigations of EBUS-TBNI into the pulmonary artery, have consistently shown that pulmonary arterial puncture using a 21-22G needle is well tolerated and does not result in significant hemorrhage[15-17]. These earlier reports, together with the absence of bleeding complications in the present study, support the procedural safety of EBUS-based vascular access for organoid transplantation. Nevertheless, continued vigilance and further evaluation in longer-term studies will be essential as this technique advances toward clinical translation.

From a clinical perspective, airway organoid transplantation could serve as a bridge therapy or adjunct to lung transplantation. Patients with IPF and COPD as well as those with chemotherapy or radiation-induced lung injury, may particularly benefit from regenerative interventions that target the peripheral lung parenchyma [1–3]. Moreover, patients on lung transplant waiting lists could potentially receive airway organoid infusions to stabilize lung function until suitable donor lungs become available. In the longer term, repeated vascular delivery of autologous or engineered organoids may reduce disease progression and improve survival.

Despite these promising findings, several additional limitations should be acknowledged. First, the observation period was limited to 7 days, and long-term survival, integration, and functional recovery of engrafted cells remain unknown. Second, although immunosuppression was used, immune responses may differ in a clinical setting, particularly with allogeneic or xenogeneic transplantation. Optimization of immunosuppressive regimens may be required for translation. Third, while we demonstrated differentiation into alveolar epithelial lineages, the functional capacity of engrafted organoids such as

contribution to gas exchange was not directly tested. Future studies should address these limitations by extending follow-up periods, performing functional assays of gas exchange and lung mechanics.

Ultimately, if safety and efficacy are confirmed, EBUS-guided PA injection of airway organoids may provide a clinically feasible regenerative therapy for end-stage lung diseases, offering an urgently needed alternative to donor organ transplantation.

Declarations

Disclosure statement:

HO was supported in part by Uehara Memorial Foundation. TK was supported in part by Uehara Memorial Foundation. MST received research funding unrelated to this work from AstraZeneca, Bayer, Bristol-Myer Squibb and Sanofı, and consultation fees from AstraZeneca, Amgen, Abbvie, Daiichi Sankyo, Regeneron, Sanofı. KY research funding from Siemens, Zidan Medical Inc. and OKF Technology, and consultant fee from Olympus America Inc., Medtoronic and Johnson & Johnson Enterprise Innovation. Other authors declare no conflicts of interest.

Competing Interests

HO was supported in part by Uehara Memorial Foundation. TK was supported in part by Uehara Memorial Foundation. MST received research funding unrelated to this work from AstraZeneca, Bayer, Bristol-Myer Squibb and Sanofı, and consultation fees from AstraZeneca, Amgen, Abbvie, Daiichi Sankyo, Regeneron, Sanofı. KY research funding from Siemens, Zidan Medical Inc. and OKF Technology, and consultant fee from Olympus America Inc., Medtoronic and Johnson & Johnson Enterprise Innovation. Other authors declare no conflicts of interest.

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Author Contribution

HO: Conceptualization, Methodology, Investigation, Animal surgery, Data acquisition, Data curation and analysis, Writing - original draft, Writing – review and editing.YF: Methodology, Investigation, Animal surgery, Data acquisition, Data curation and analysis, Writing – review.TK: Methodology, Investigation, Animal surgery, Data acquisition, Writing – review and editing.NB: Methodology, Investigation, Animal surgery, Data acquisition, Data curation and analysis, Writing - original draft, Writing – review and

editing.YS: Methodology, Investigation, Animal surgery, Data acquisition, Writing – review and editing.SK: Methodology, Investigation, Animal surgery, Data acquisition, Writing – review and editing.YH: Methodology, Investigation, Animal surgery, Data acquisition, Writing – review and editing..FY: Investigation, Animal surgery, Data acquisition, Writing – review and editing.TY: Investigation, Animal surgery, Data acquisition, Writing – review and editing.KN: Investigation, Animal surgery, Data acquisition, Writing – review and editing.HH: Investigation, Animal surgery, Data acquisition, Writing – review and editing.AE: Investigation, Data acquisition, Writing – review and editing.KK: Investigation, Data acquisition, Writing – review and editing, Illustration.TA: Methodology, Writing – review and editing.YM: Methodology, Writing – review and editing.MT: Methodology, Investigation, Pathological analysis, Data acquisition, Data curation and analysis, Writing - original draft, Writing – review and editing, Administrative SupportKY: Conceptualization, Methodology, Writing – review and editing, Administrative Support, Supervision

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Figures

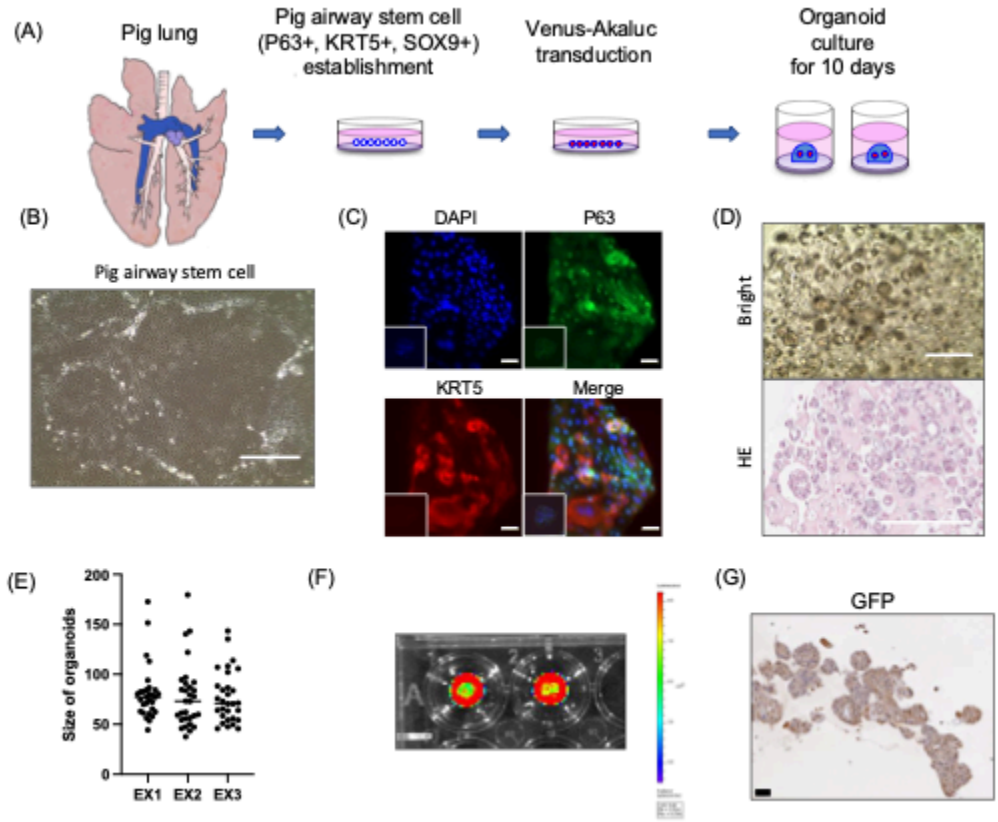

Figure 1

Establishment and characterization of pig airway stem cell-derived organoids with Venus-Akaluc labeling.

(A) Overview of the workflow for establishing pig airway stem cells, generating airway organoids, and Venus-Akaluc transduction.

(B) Representative image of established pig airway stem cells. Scale bar represents 400 μm.

(C) Immunofluorescence staining showing DAPI (blue), P63 (green), and KRT5 (red) expression in airway stem cells. Scale bars represent 50 μm.

(D) Representative images of airway organoids derived from pig airway stem cells: bright-field microscopy (top) and hematoxylin and eosin (H&E) staining (bottom). Scale bars represent 400 μm.

(E) Quantitative analysis of organoid size after 10 days of culture.

(F) Bioluminescence detection of Akaluc signal in cultured organoids using the Xenogen IVIS Spectrum system.

(G) Representative immunohistochemical images showing GFP of Venus-Akaluc transduced organoids. Scale bars represent 50 μm.

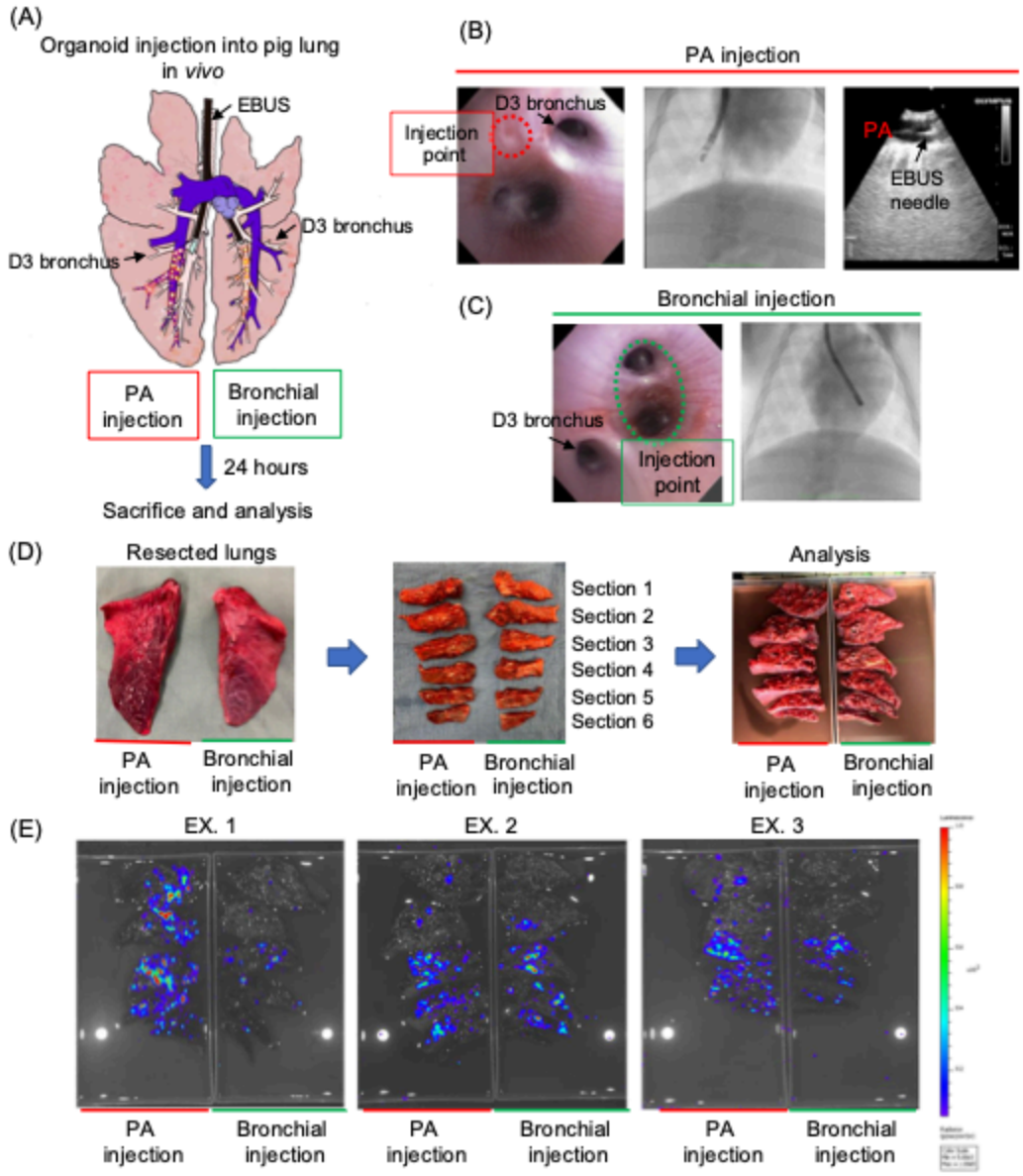

Figure 2

Comparison of pulmonary arterial (PA) and bronchial injection of airway organoids in vivo.

(A) Schematic illustration of airway organoid injection into the pig lung.

(B) Representative intra-procedural images during PA injection Left panel: bronchoscopy; middle panel: fluoroscopy; right panel: EBUS.

(C) Representative intra-procedural images during bronchial injection. Left panel: bronchoscopy; right panel: fluoroscopy.

(D) Macroscopic image of the resected pig lung 24 hours after organoid injection. The bilateral lower lobes were resected, divided axially into six sections, and analyzed by the Xenogen IVIS Spectrum system.

(E) Bioluminescence detection of Akaluc signals in the pig lung using the Xenogen IVIS Spectrum system.

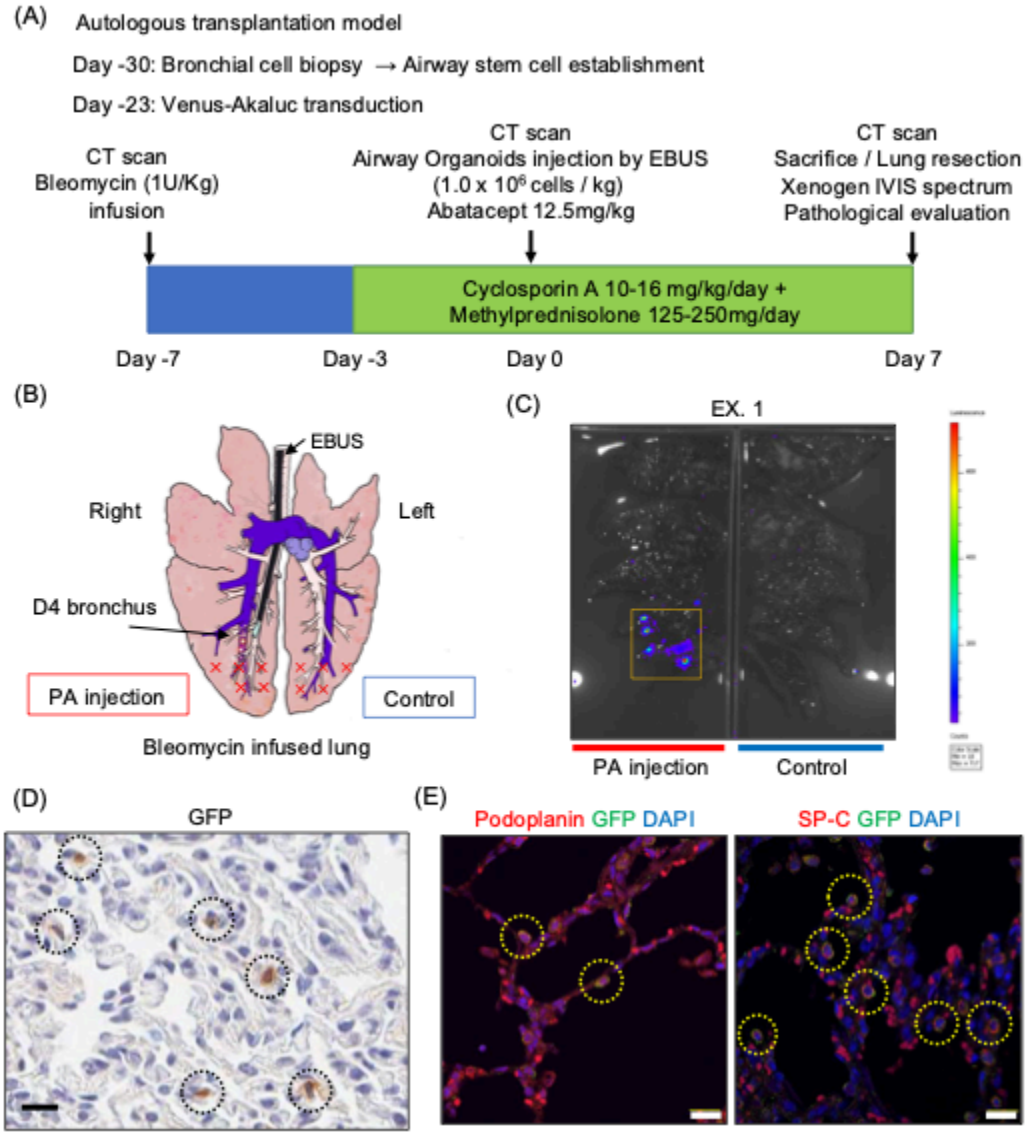

Figure 3

Autologous organoid transplantation and evaluation in the 7-day survival model.

(A) Overview of the organoid injection procedure.

(B) Schematic illustration of autologous airway organoid injection in the 7-day survival model.

(C) Bioluminescence detection of Akaluc signals in the pig lung on day 7 using the Xenogen IVIS Spectrum system.

(D) Representative immunohistochemical images of GFP-positive organoids following PA injection. Scale bars represent 20 μm.

(E) Double immunofluorescence staining showing that a subset of GFP-positive cells co-expressed podoplanin (alveolar type I cell marker) and SP-C (alveolar type II cell marker). Scale bars represent 20 μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 260419Pigairwaystemcellfiguressupplimentaryfigures.pdf
- Movie1.mp4
- Movie2.mp4