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# Fibroblasts Positively Influence Lung Progenitor Cells In Organoids

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Master thesis, Bioanalytics

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## INTRODUCTION

Lung progenitor cells, alveolar type II (AT2) epithelial cells, play an important role in lung development due to their ability to transdifferentiate into alveolar type I (AT1) cells and rapid expansion after injury [1]. Although little is known about the underlying mechanisms that control the AT2 cell activation, mesenchymal cells as well as growth factors are known to closely associate with alveolar epithelial cells during development and regeneration. However, the exact interaction mechanism of fibroblasts with lung progenitor cells remains unclear [2]. Therefore, the generation of robust and reproducible 3D organoid models is of great interest to better understand key factors responsible for alveolar growth and regeneration. Specifically the impact of the transcription factor 21 (Tcf21) on lung progenitor cells was further studied.

## METHODS

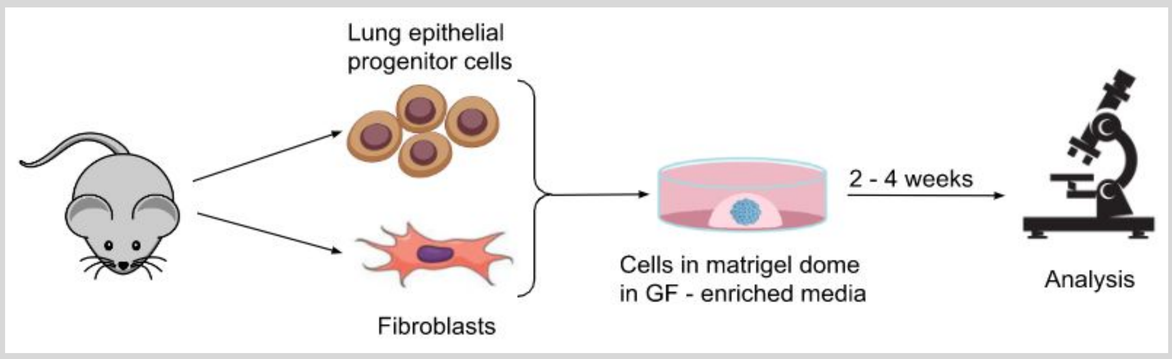


**Figure 1** Organoids were generated from purified lung epithelial progenitor cells combined with separated fibroblasts. The cells were embedded in a dome of matrigel and surrounded by growth factor-enriched media. The analysis was performed after 2 - 4 weeks in culture.

## RESULTS

Lung organoid systems were optimized through the variation of the dome plating methods, numbers of fibroblasts, and concentrations and combinations of GFs.

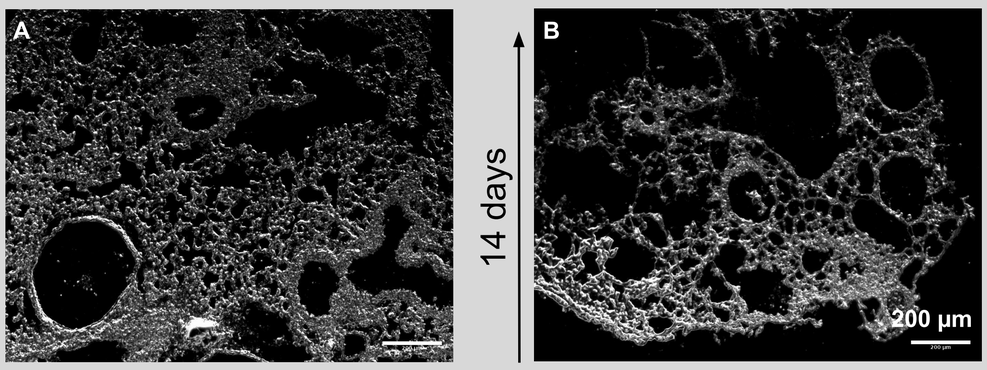


**Figure 2** Bright field (BF) images of histological sections confirmed the successful generation of organoids through the similarity of the structure compared to a mouse lung lobe A: section of a mouse post-caval lobe, B: section of a lung organoid after 2 weeks in culture

The development of the organoids was investigated using the rainbow reporter system. When Cre recombinase gets activated individual cells randomly and permanently express one of four fluorescence protein colors allowing visualization of clonally expanded cells.

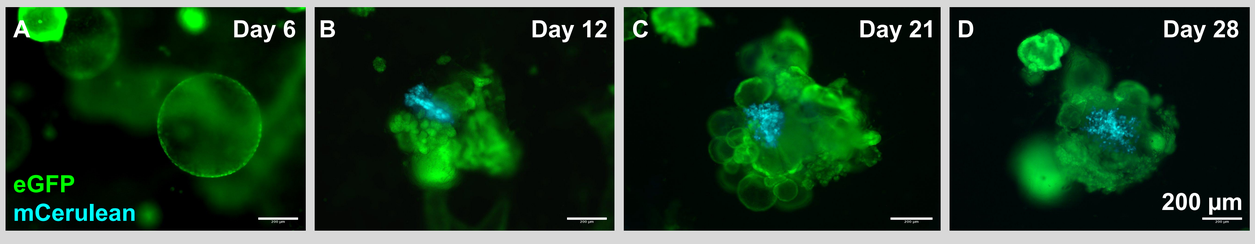


**Figure 3** Development of an organoid over 28 days using the rainbow reporter system. Because of the permanent DNA recombination, all cerulean$^+$ cells originate from the same parenteral cell

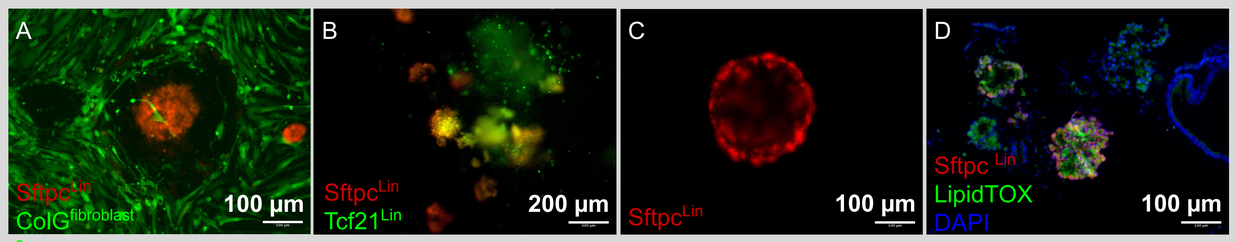


**Figure 4** Investigation of the impact of fibroblasts on the formation of lung organoids A: unseparated ColG$^+$ fibroblasts surrounding an organoids derived from tdTomato$^+$ lung progenitor cells, B: Tcf21$^+$ lipofibroblasts incorporating into the organoids: C: negative control, lung progenitor cells cultured without fibroblasts, D: green LipidTOX and DAPI staining of sectioned organoids cultured with unlabeled fibroblasts

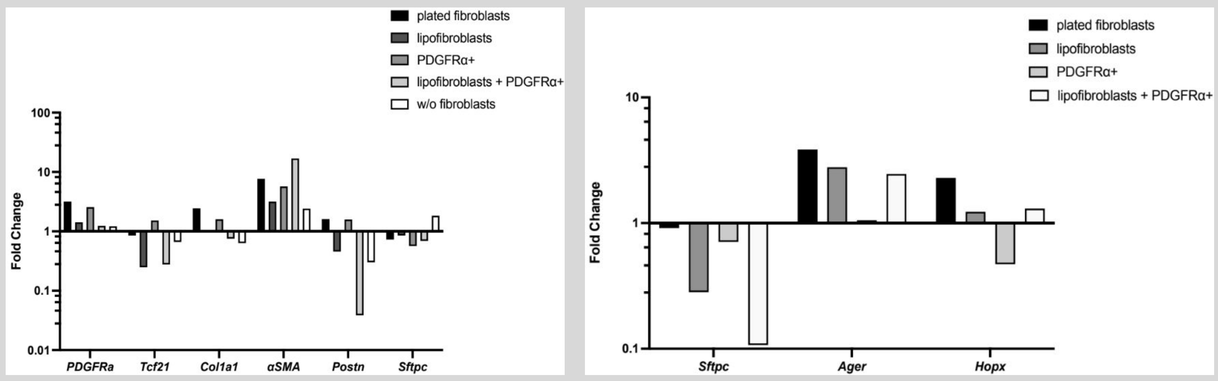


**Figure 5** Gene expression analysis of organoids cultured with different types of fibroblasts A: average fold change of *PDGFRa*, *Tcf21*, *Col1a1*, *αSMA*, *Postn*, *Sftpc* compared to perinatal (P) day 1 lungs, B: average fold change of *Sftpc*, *Ager*, and *Hopx* compared to cultures without fibroblasts

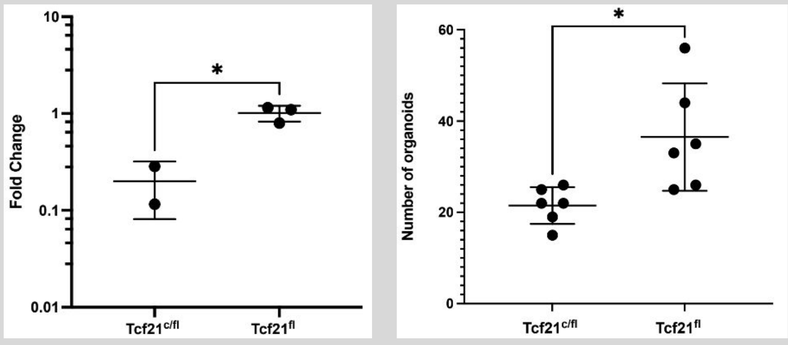


**Figure 6** Investigation of the impact of Tcf21 knock-out (KO) fibroblasts on the growth of lung organoids A: Depletion efficiency of Tcf21 in KO (Tcf21$^{c/fl}$) vs. controls (Tcf21$^{fl}$), B: significantly reduced number of organoids when cultured with Tcf21 KO fibroblasts

## DISCUSSION

Fluorescence analyses visualized the close association of fibroblasts, specifically Tcf21$^+$ lipofibroblasts, with lung progenitor cells. The organoids showed an alveoli-shaped structure, whereas when cultured without fibroblasts, organoids remained in a roundish shape, suggesting less differentiation of AT2 cells. These observations give a first indication of a positive interaction between fibroblasts and lung progenitor cells. An upregulation of the AT2 marker *Sftpc* in organoids without fibroblasts, but downregulation with fibroblasts compared to P1 lungs suggests a transdifferentiation of AT2 into AT1 cells. The downregulation of *Sftpc* and upregulation of AT1 marker *Ager* and *Hopx* in organoids with fibroblasts compared to those without fibroblasts supported these assumptions. Furthermore, an efficient depletion of Tcf21 led to a significantly decreased number of organoids compared to controls, suggesting Tcf21 to be a key factor in lung organoid formation. However, more experiments are needed to confirm this hypothesis.

## REFERENCES

[1] A. M. Olajuyin, X. Zhang, and H.-L. Ji, "Alveolar type 2 progenitor cells for lung injury repair," *Cell death discovery*, vol. 5, p. 63, 2019, doi: 10.1038/s41420-019-0147-9.

[2] M. G. Ushakumary, M. Riccetti, and A.-K. T. Perl, "Resident interstitial lung fibroblasts and their role in alveolar stem cell niche development, homeostasis, injury, and regeneration," *Stem cells translational medicine*, vol. 10, no. 7, pp. 1021–1032, 2021, doi: 10.1002/sctm.20-0526.