

Innovative Approaches: Lung Cancer Organoid Modeling, Verification, and Clinical Utility

Department of Internal Medicine, Chungnam National University Hospital

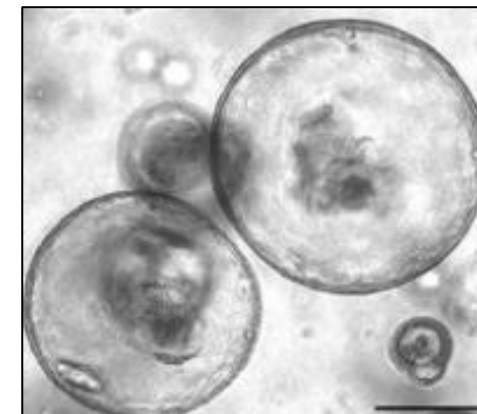
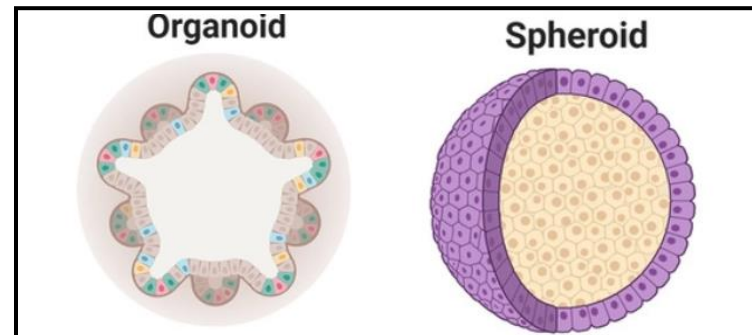
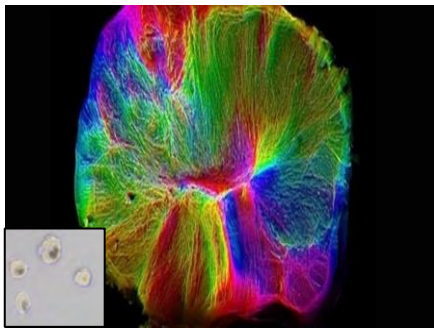
Chaeuk Chung M.D./Ph.D.

Organoid (mini-organ)

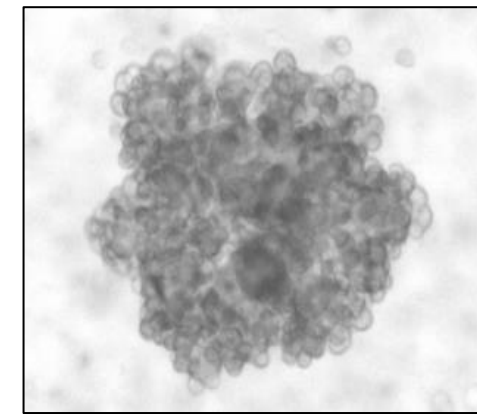
- **ES cell** or **iPS cell**, **Adult stem cell**
 - Self organize in **3D culture** (self renewal, differentiation)
- **Miniaturized and simplified version of an organ**
- Contain the **multiple cell types** of a particular organ

Lung organoids

Organoid (Brain)

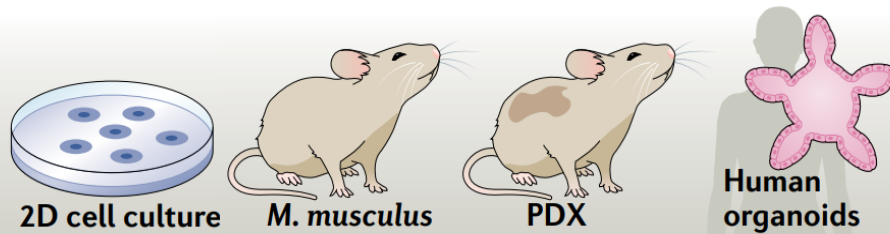


Airway organoid



Alveolar organoid
(Alveolosphere)

Pro and Cons of Organoid



2D cell culture

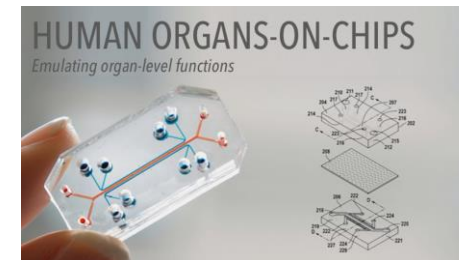
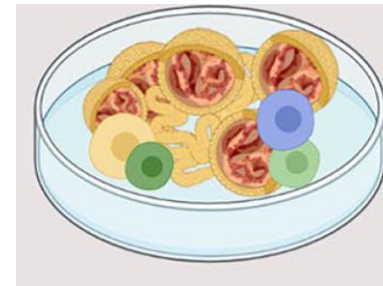
M. musculus

PDX

Human organoids

| | 2D cell culture | <i>M. musculus</i> | PDX | Human organoids |
|---|-----------------|--------------------|-----|-----------------|
| Ease of establishing system | ✓/✗ | ✓ | ✓ | ✓ |
| Ease of maintenance | ✓ | ✓ | ✓ | ✓ |
| Recapitulation of developmental biology | ✗ | ✓ | ✗ | ✓ |
| Duration of experiments | ✓ | ✓ | ✓ | ✓ |
| Genetic manipulation | ✓ | ✓ | ✗ | ✓ |
| Genome-wide screening | ✓ | ✗ | ✗ | ✓ |
| Physiological complexity | ✗ | ✓ | ✓ | ✓ |
| Relative cost | ✓ | ✓ | ✓ | ✓ |
| Recapitulation of human physiology | ✓ | ✓ | ✓ | ✓ |

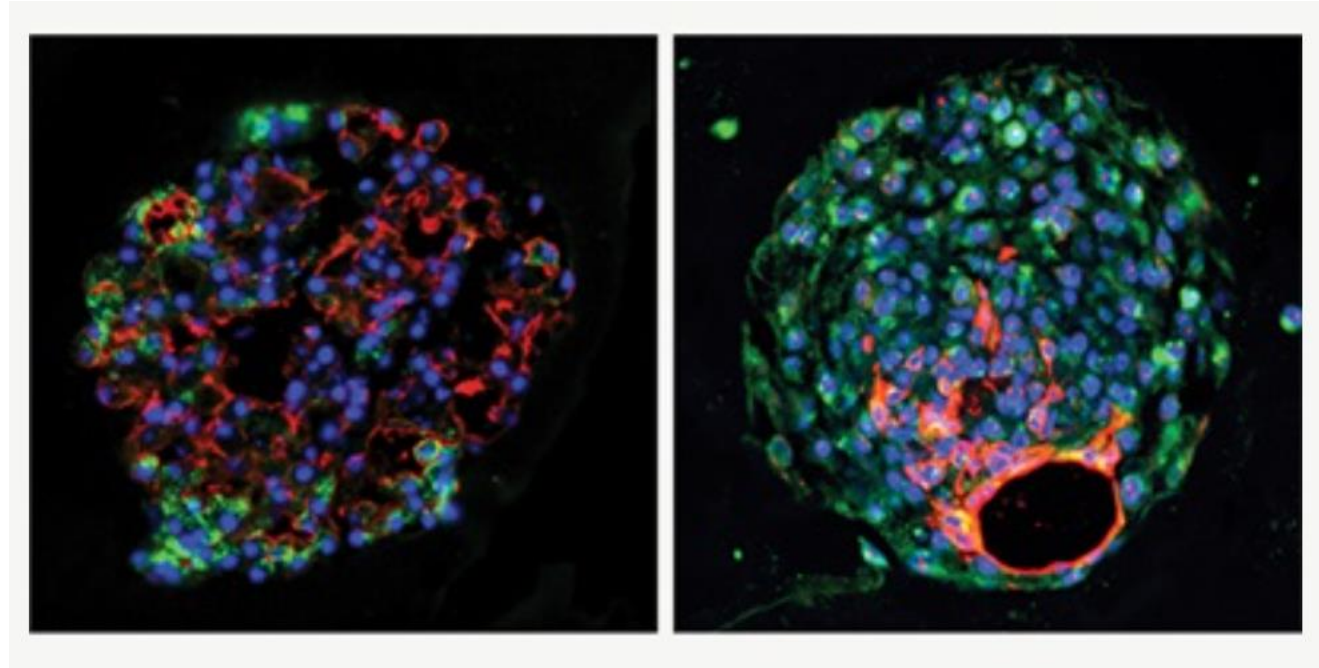
Organoid-mesenchymal cell co-culture



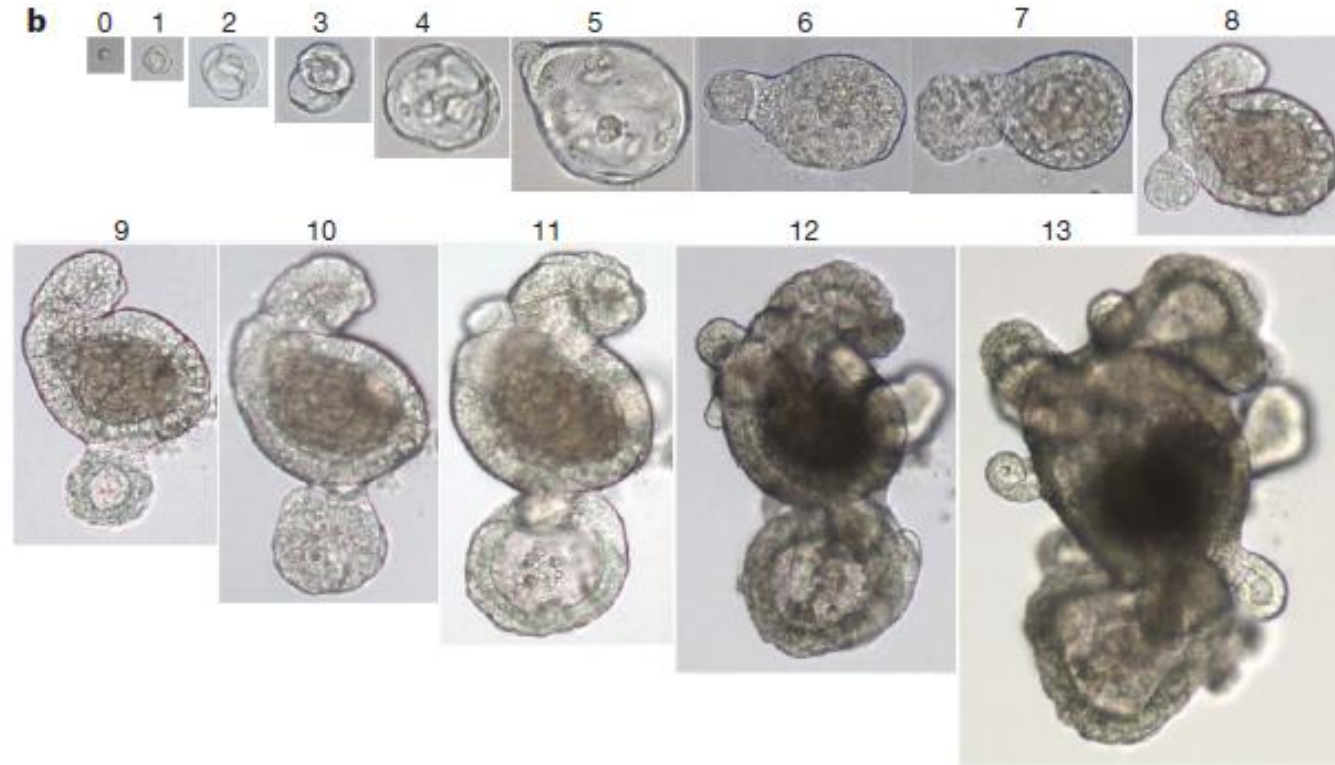
Clinical study



History of Organoid



Intestinal organoid



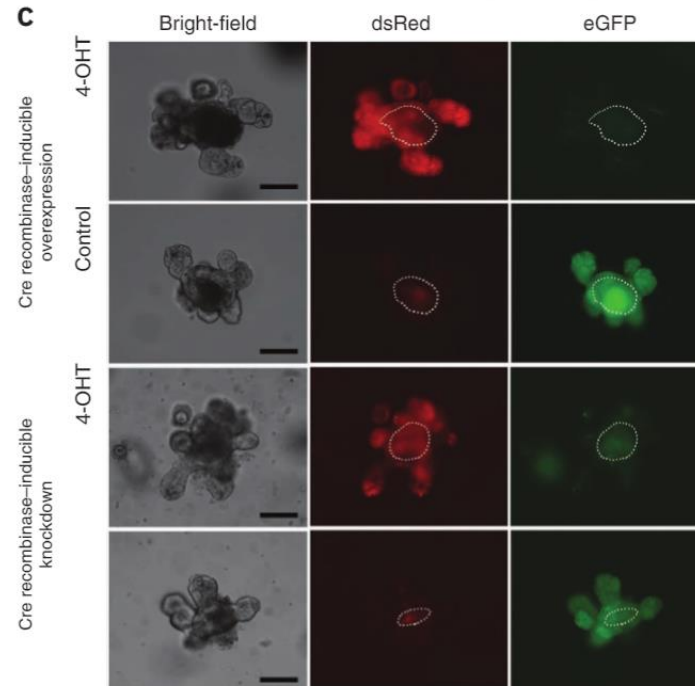
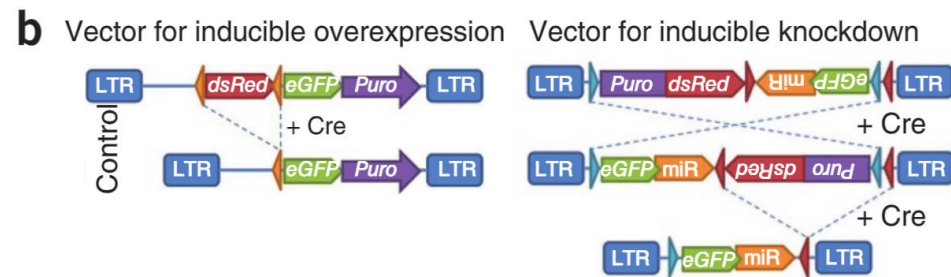
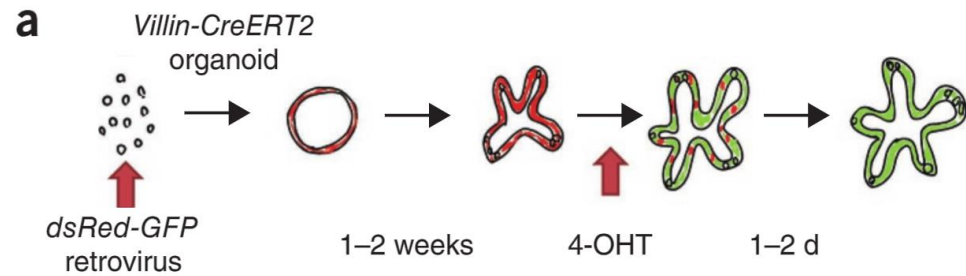
Dr. Hans Clevers
Roche, Head of Pharma Research
and Early Development



Toshiro Sato,
Keio University

- Toshiro Sato, **Single Lgr5 stem cells** build crypt-villus structures in vitro without a mesenchymal niche, Nature, 2009

Gene manipulation in Organoid



Prof. Dr. Hans Clevers
Roche, Head of Pharma Research and
Early Development



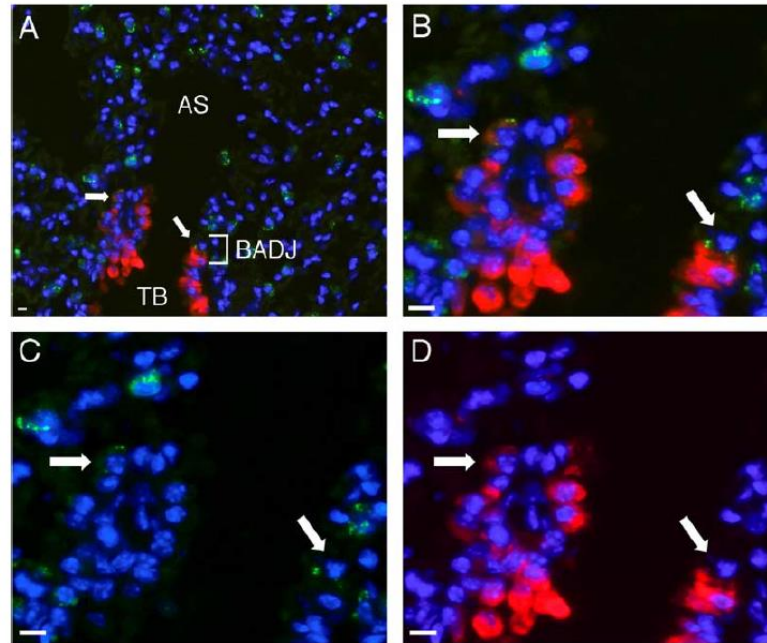
Bon-Kyoung KOO,
IBS

Lung stem cell

Cell, Vol. 121, 823–835, June 17, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.03.032

Identification of Bronchioalveolar Stem Cells in Normal Lung and Lung Cancer

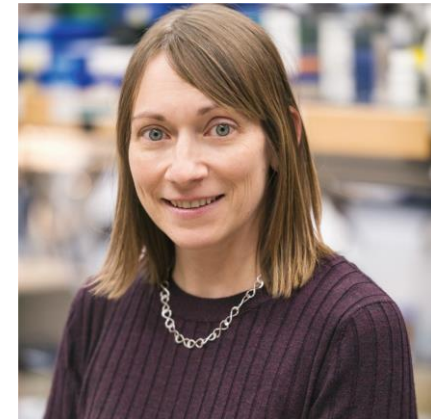
Carla F. Bender Kim,¹ Erica L. Jackson,^{1,3}
Amber E. Woolfenden,^{1,2} Sharon Lawrence,¹
Imran Babar,² Sinae Vogel,¹ Denise Crowley,¹
Roderick T. Bronson,⁴ and Tyler Jacks^{1,2,*}
¹Center for Cancer Research and Department
of Biology
²Howard Hughes Medical Institute
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139



Cell, Vol. 121, 823–835, June 17, 2005



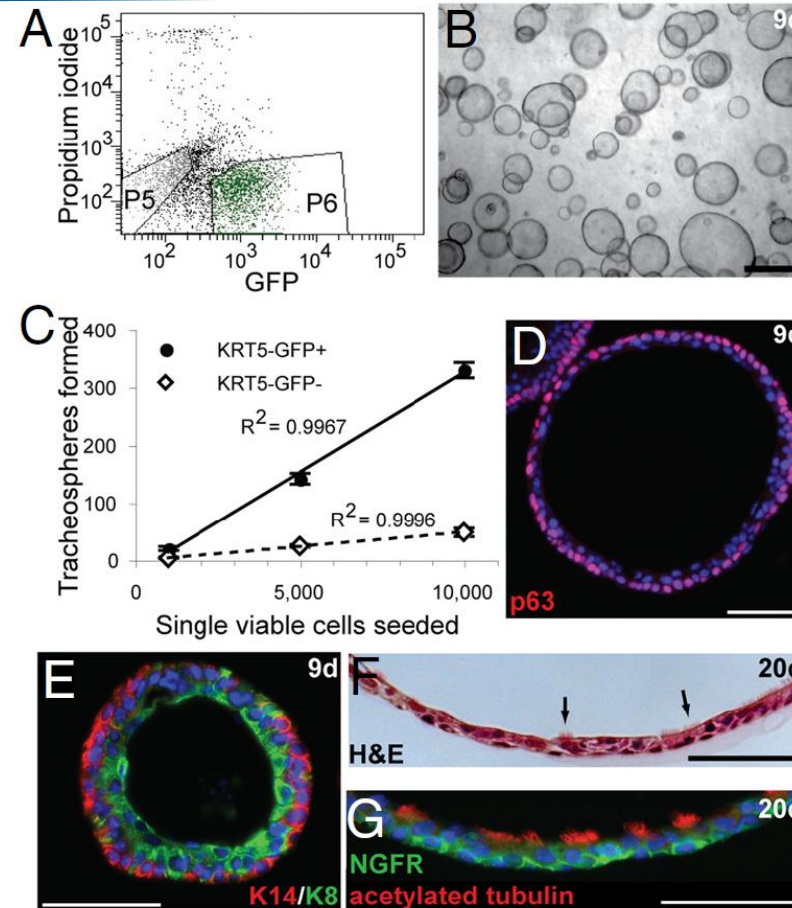
Tyler Jacks, MIT



Carla Kim,
Harvard University

Lung organoid

- The first organoids derived from mouse tracheal basal cells were called **tracheospheres**.
- Tracheal stem cell: Basal cells expressing Trp-63 (p63) and **cytokeratins 5 (Krt5)** and Krt14 (Rock et al., 2009).



Brigid. M. Hogan
 Duke university

Single viable KRT5-GFP BCs (P6) or KRT5-GFP (P5) tracheal epithelial cells

➤ J Clin Invest. 2013 Jul;123(7):3025-36. doi: 10.1172/JCI68782. Epub 2013 Jun 10.

Type 2 alveolar cells are stem cells in adult lung

Christina E Barkauskas ¹, Michael J Cronic, Craig R Rackley, Emily J Bowie, Douglas R Keene, Barry R Stripp, Scott H Randell, Paul W Noble, Brigid L M Hogan

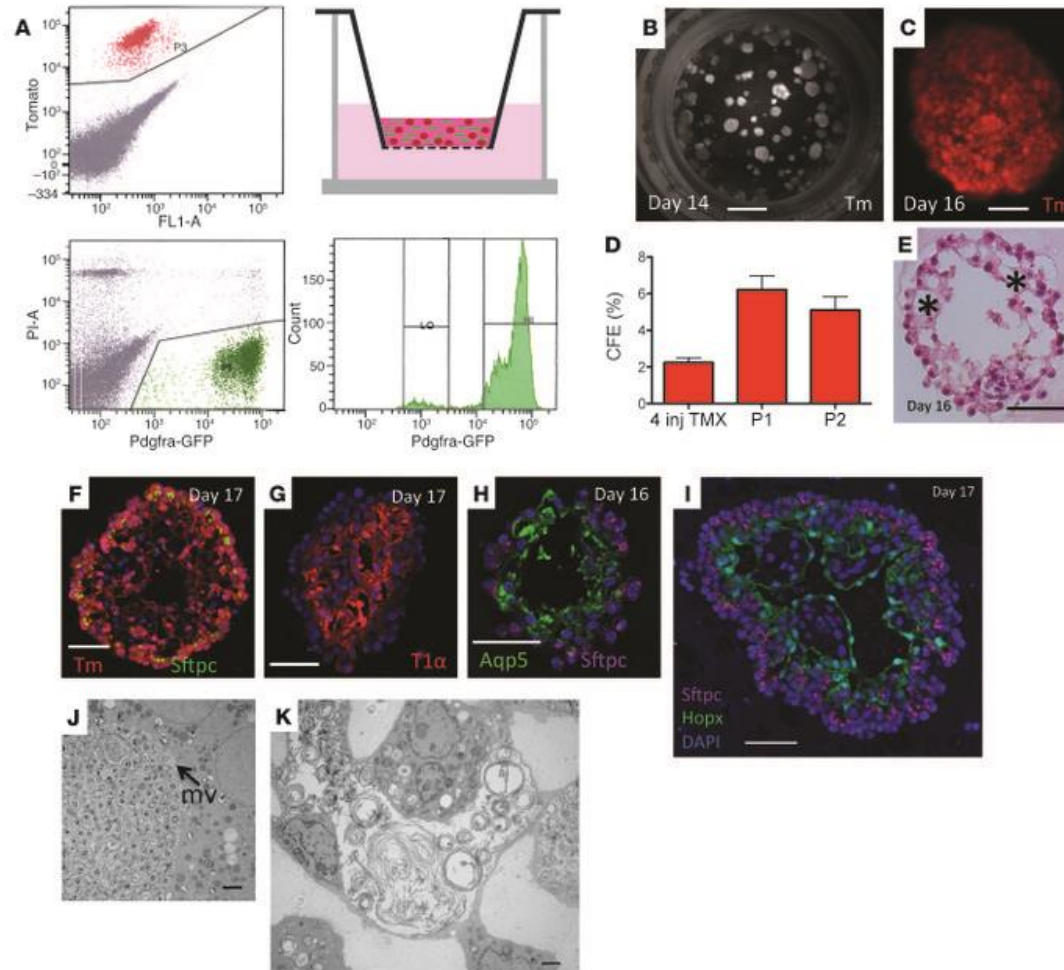
Affiliations + expand

PMID: 23921127 PMCID: [PMC3696553](#) DOI: [10.1172/JCI68782](#)



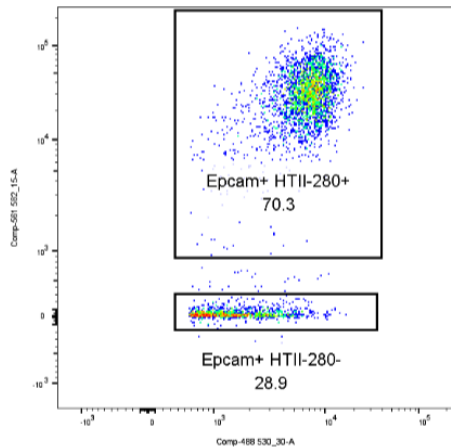
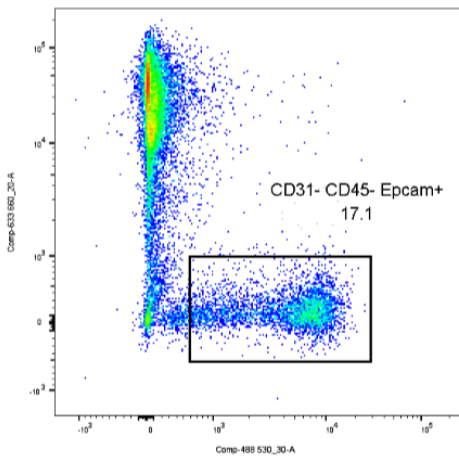
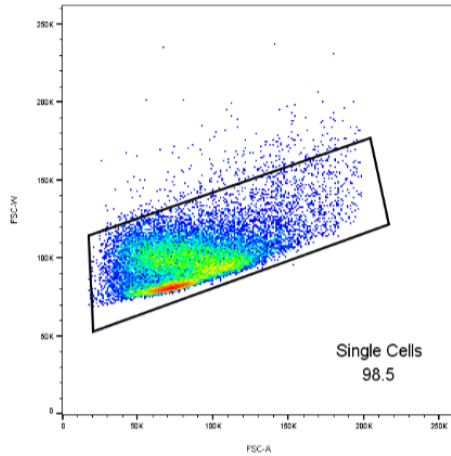
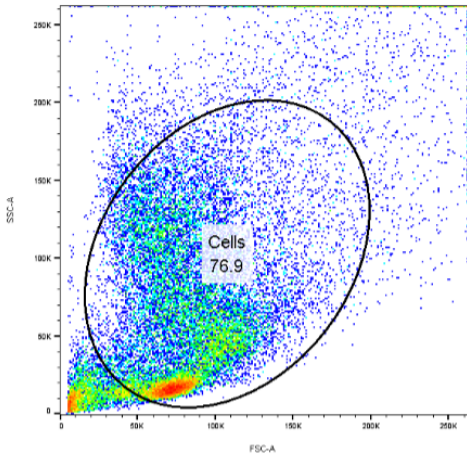
Brigid. M. Hogan
Duke university

Type 2 alveolar cells are stem cells in adult lung



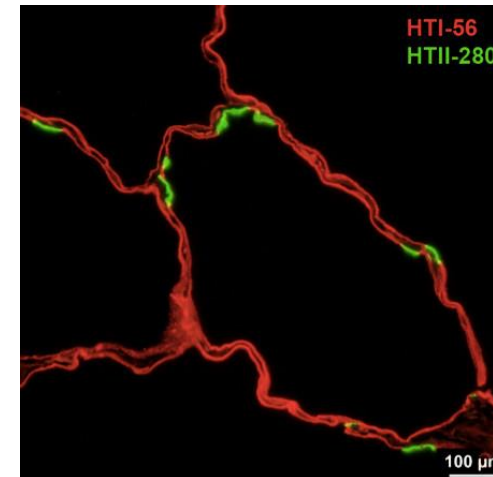
Alveolar stem cell: HTII-280 + cell

Sorting primary cells for HTII-280 and Epcam (CD31- CD45- Epcam+)



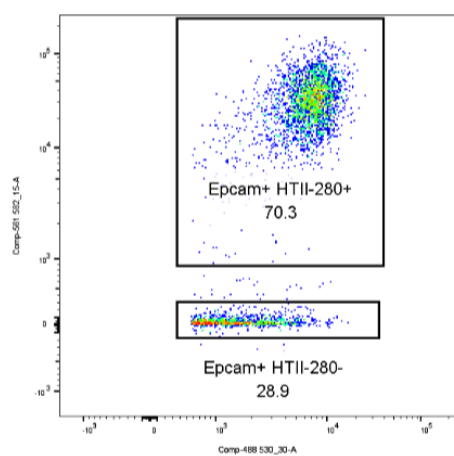
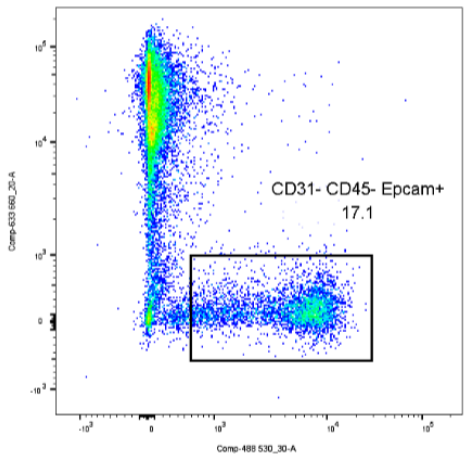
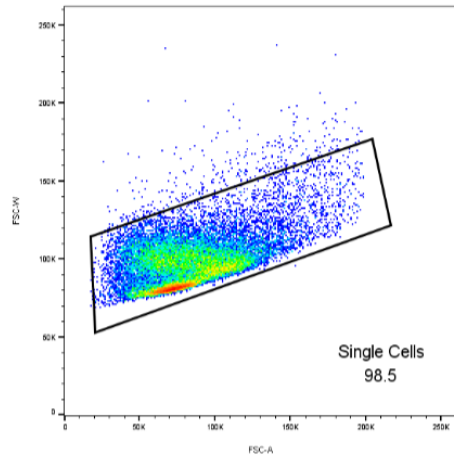
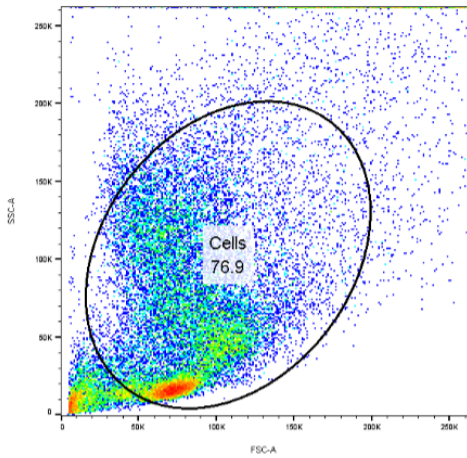
CD31- CD45- Epcam+ HTII-280+ (AT2) cells can be used to produce alveolar organoids

HTII-280: a Biomarker Specific to the Apical Plasma Membrane of Human Lung Alveolar Type II Cells
: [J Histochem Cytochem.](#) 2010 Oct; 58(10): 891–901.



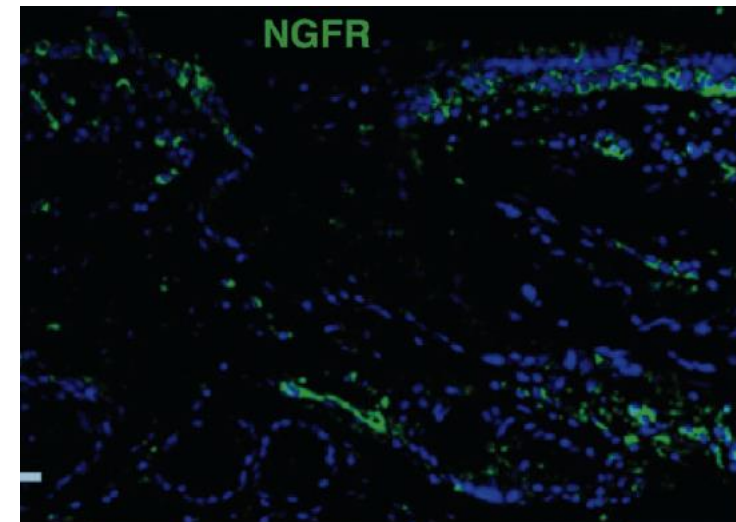
Airway stem cell: HTII-280 (-) cell

Sorting primary cells for HTII-280 and Epcam (CD31- CD45- Epcam+)

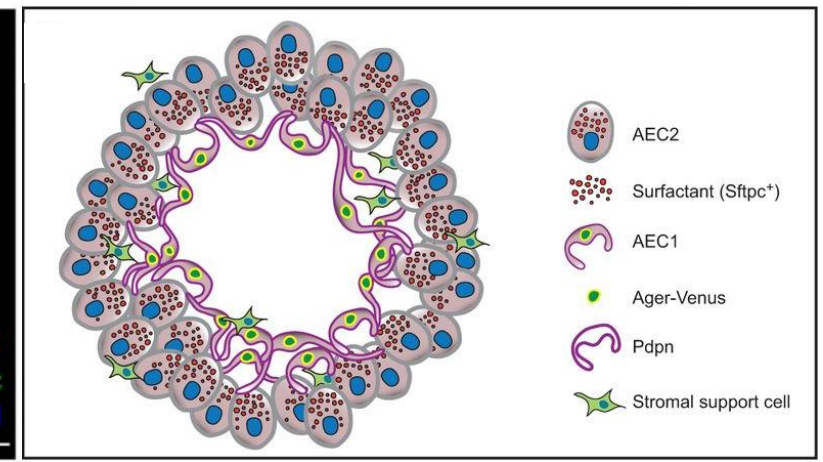
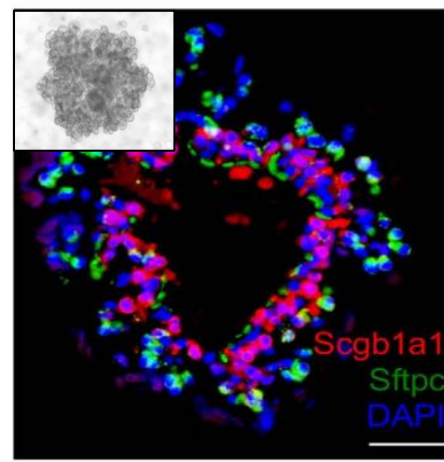
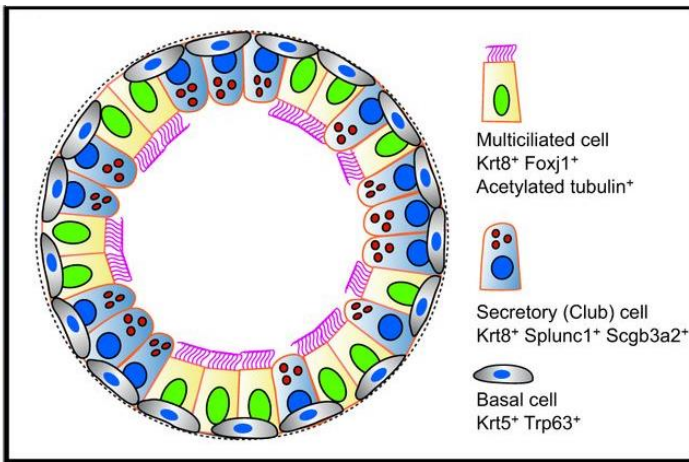
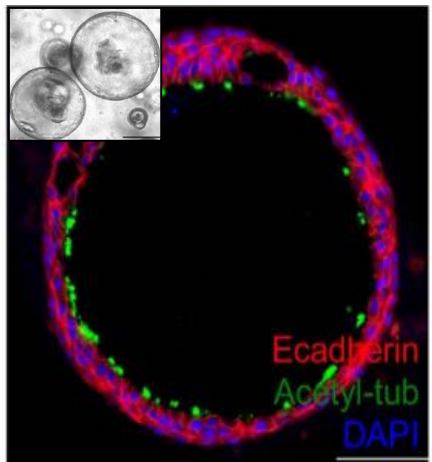
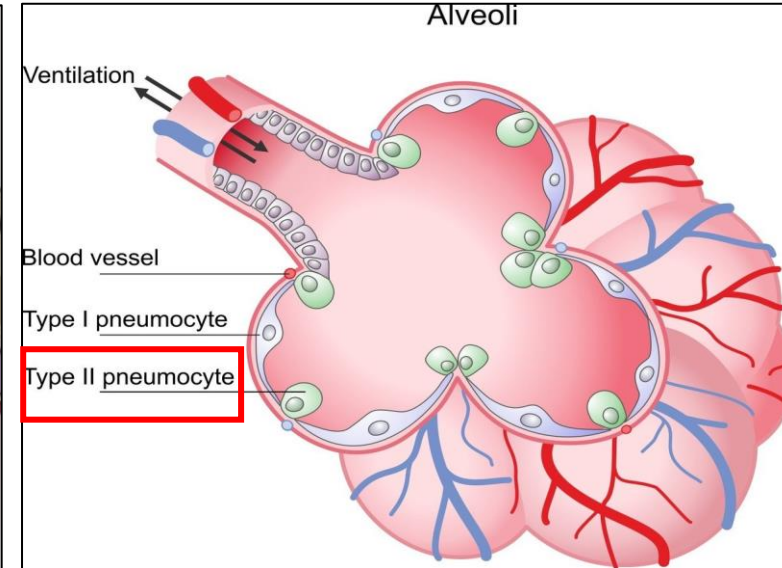
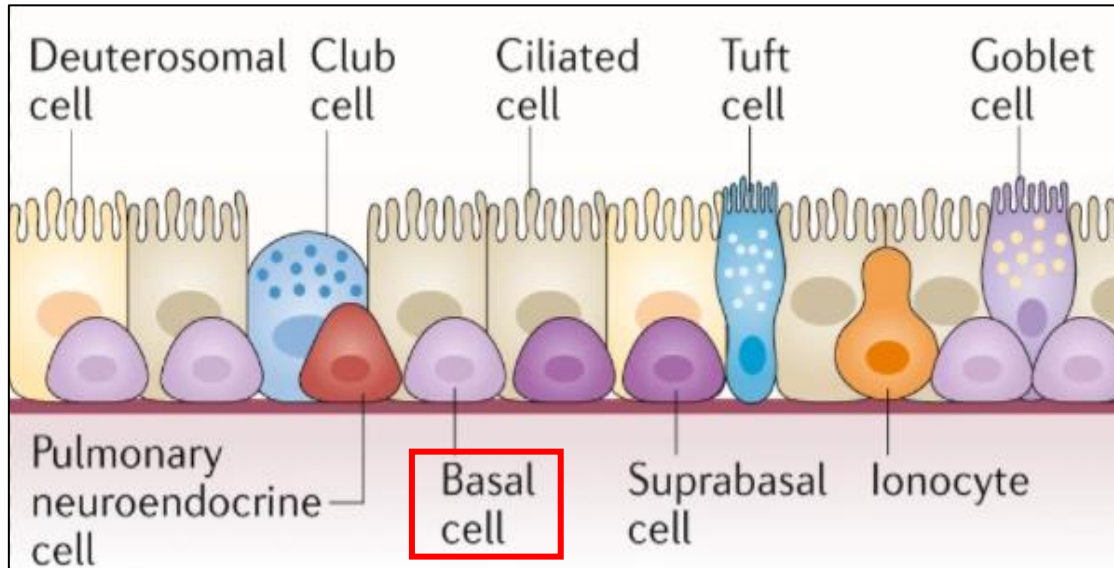


CD31- CD45- Epcam+ HTII-280- (basal cells, club cells, etc.) can be used to produce airway organoids

NGFR(Nerve Growth Factor Receptor) for sorting basal cells



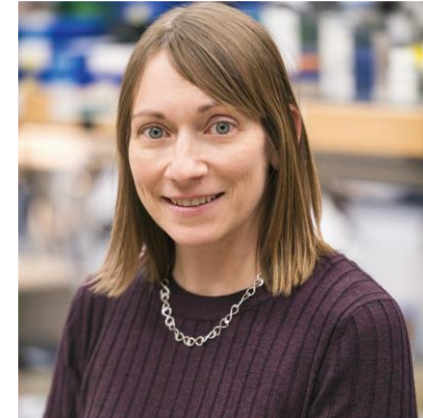
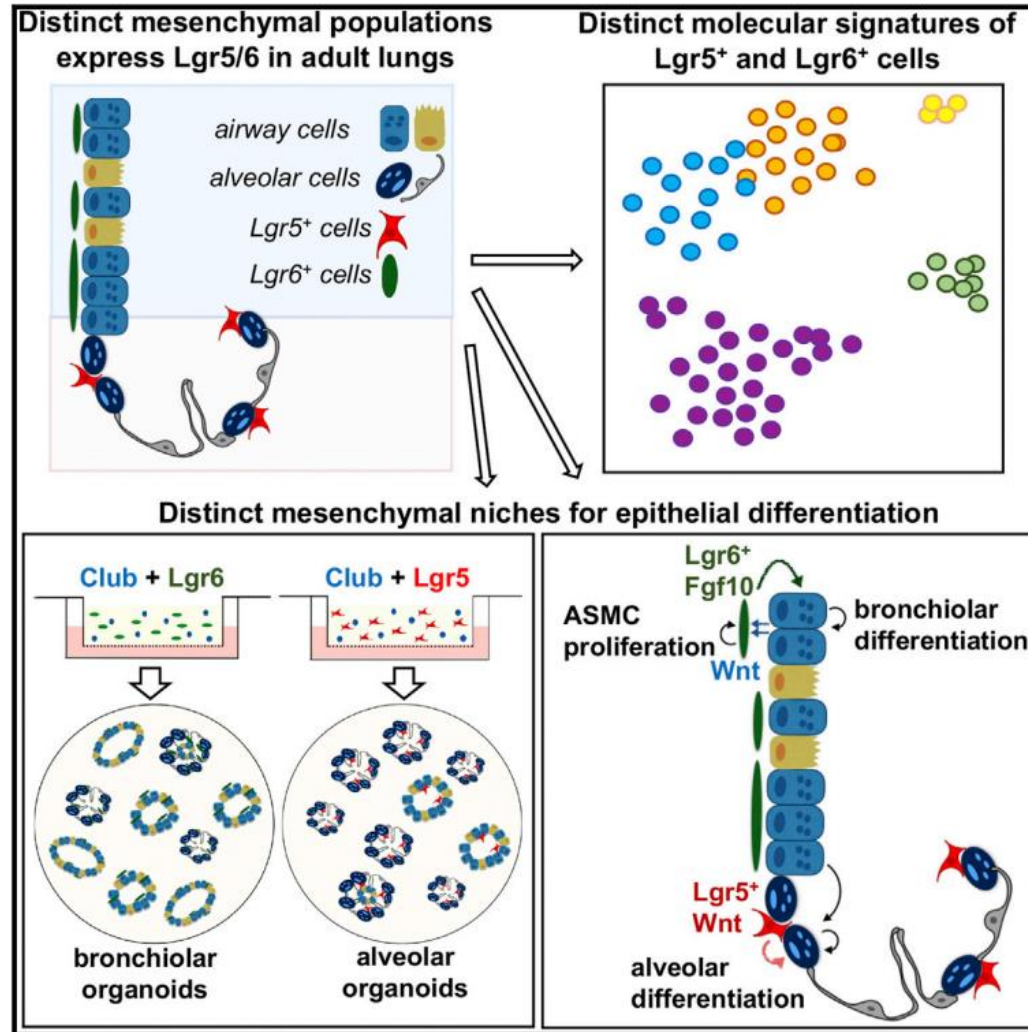
Lung organoid



Airway organoid: Basal cell

Alveolar organoid : Type 2 pneumocyte

The mesenchymal cell determines the fate of lung organoid

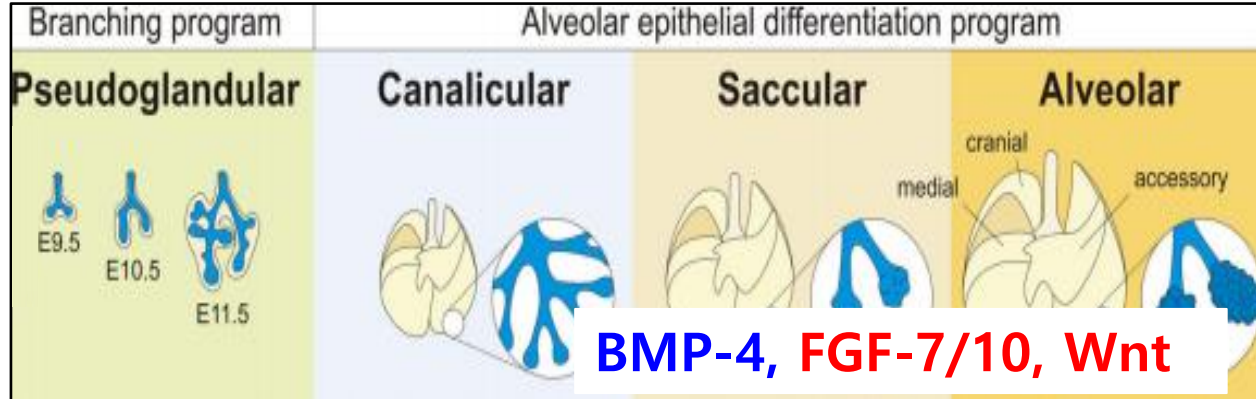


Clara Kim,
Harvard University

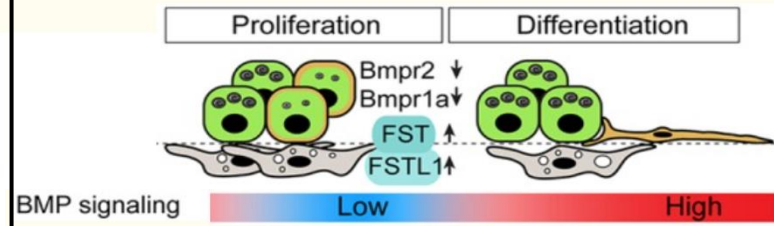


Joo-Hyeon Lee
Cambridge university

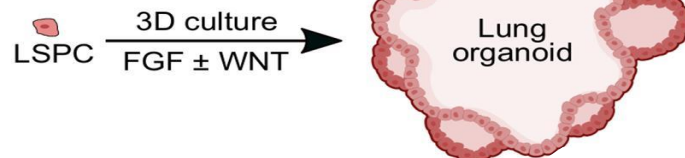
Organoid culture media & lung development, mesenchymal cells



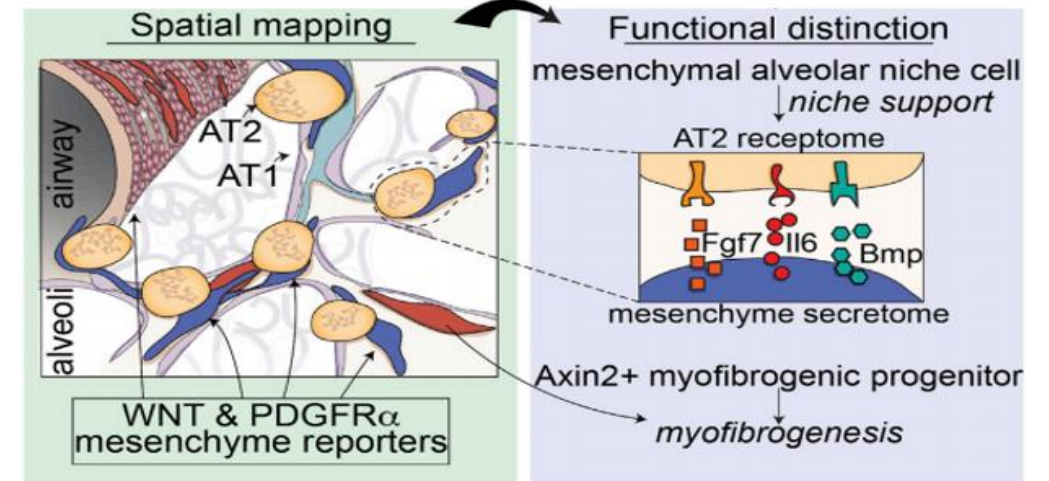
Noggin(BMPi)



FGF7/10, Wnt



Lung mesenchymal cells



FGF7, BMP, IL-6 signaling defines the alveolar niche secretome: receptome

Zepp et al., 2017, Cell 170, 1134–1148

Int. J. Mol. Sci. 2016, 17(1)

Dev Dyn. 2015 March ; 244(3): 342–366.

Media components for culture of human airway organoids

| Media component | Signalling pathway | | Supplier | Stock Concentration | Final Concentration | Amount/ 50ml |
|----------------------------------|--------------------------|-------|----------------------------|---------------------|---------------------|--------------|
| | activation | block | | | | |
| Advanced DMEM +++ (see below) | Base medium | | Fisher Scientific 11510436 | N/A | N/A | Up to 50ml |
| R-Spondin 1 (conditioned medium) | Wnt/b-catenin signalling | | CSCI core facility | N/A | 10% | 5ml |
| B27 supplement | a.o. insulin signalling | | Thermofisher 17504044 | 50x | 1x | 1ml |
| FGF 7 | FGFR2b signalling | | Peprtech 100-19 | 100µg/ml | 100 ng/ml | 50µl |
| FGF 10 | FGFR2b signalling | | Peprtech 100-25-100 | 100µg/ml | 100ng/ml | 50µl |
| Noggin | TGF-b signalling | | Peprtech 250-38-100 | 100µg/ml | 100ng/ml | 50µl |
| N-Acetylcysteine | Antioxidant | | Sigma A9165 | 500mM | 1mM | 100µl |
| Nicotinamide | Co-enzyme precursor | | Sigma N0636 | 2M | 10mM | 250µl |
| SB431542 | TGF-b signalling | | Tocris 1614/10 | 10mM | 10uM | 50µl |
| *SB202190 | P38 MAPK signalling | | Sigma S7067 | | 500nM | 5µl |

Advanced DMEM +++

Add the following to Adv DMEM (500 mL)

| | | | | | |
|---------------------------|-------------|----------------------|---------|-------|-------|
| Hepes | Buffer | Invitrogen 15630-080 | 1M | 10mM | 5.5ml |
| Penicillin / Streptomycin | Antibiotics | CSCI core facility | 100U/ml | 1U/ml | 5.5ml |
| L/glutamine | Amino acid | CSCI core facility | 200mM | 2mM | 5.5ml |

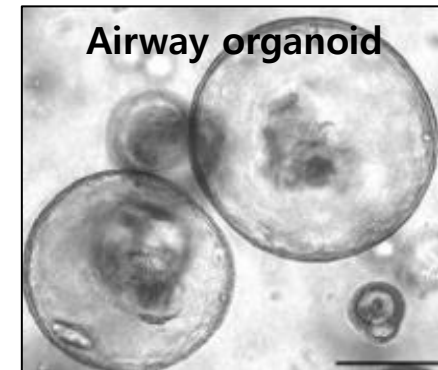
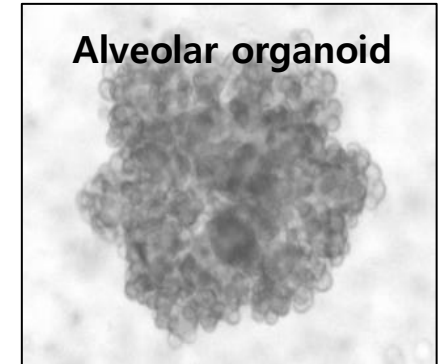
Add to single cells (after isolation or splitting for the first 48 hrs of culture)

| | | | | | |
|---------|-----------------|---------------------------------|------|------|-----------|
| Y-27632 | ROCK signalling | SM02-100 (Cambridge Bioscience) | 10mM | 10µM | 1 to 1000 |
|---------|-----------------|---------------------------------|------|------|-----------|



Lung organoid culture media

| Reference | Intestinal organoid media (Sato et al. 2011) | Human alveolar organoid media (Nickolic et al. 2017) | Human airway organoid media (Sachs et al. 2019) |
|----------------------------|--|---|--|
| Modified media composition | R-spondin 1 WNT 3A | R-spondin1 WNT 3A or CHIR99021 | R-spondin 1 |
| | Noggin (BMPi) FGF 10 A83-01 SB202190 EGF Nicotinamide B27 Gastrin | Noggin (BMPi) FGF 7/FGF 10 A83-01 SB431542 EGF Nicotinamide B27 N-acetylcysteine | Noggin (BMPi) FGF 7/FGF 10 A83-01 SB202190 Nicotinamide B27 N-acetylcysteine |
| | ROCK inhibitor(Y27632) | | |

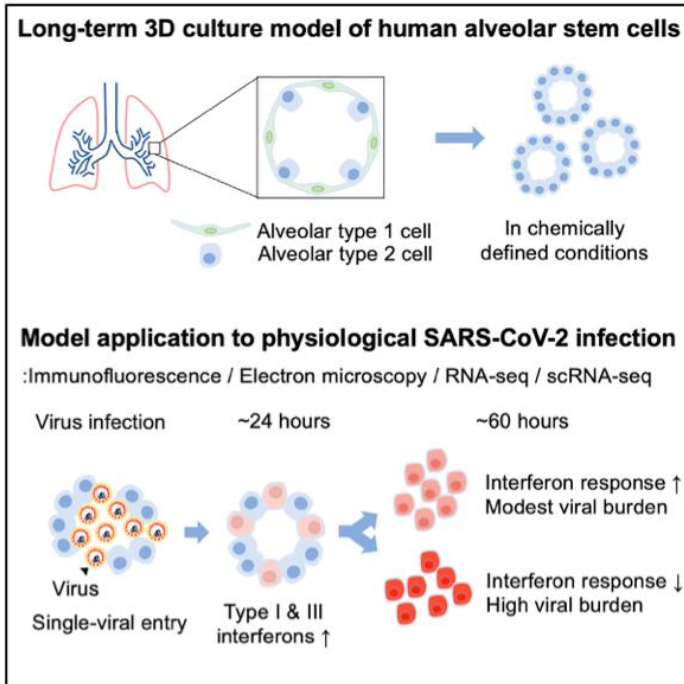


Translational Research with Lung organoid

Cell Stem Cell

Three-Dimensional Human Alveolar Stem Cell Culture Models Reveal Infection Response to SARS-CoV-2

Graphical Abstract



Authors

Jeonghwan Youk, Taewoo Kim, Kelly V. Evans, ..., Byeong-Sun Choi, Young Seok Ju, Joo-Hyeon Lee

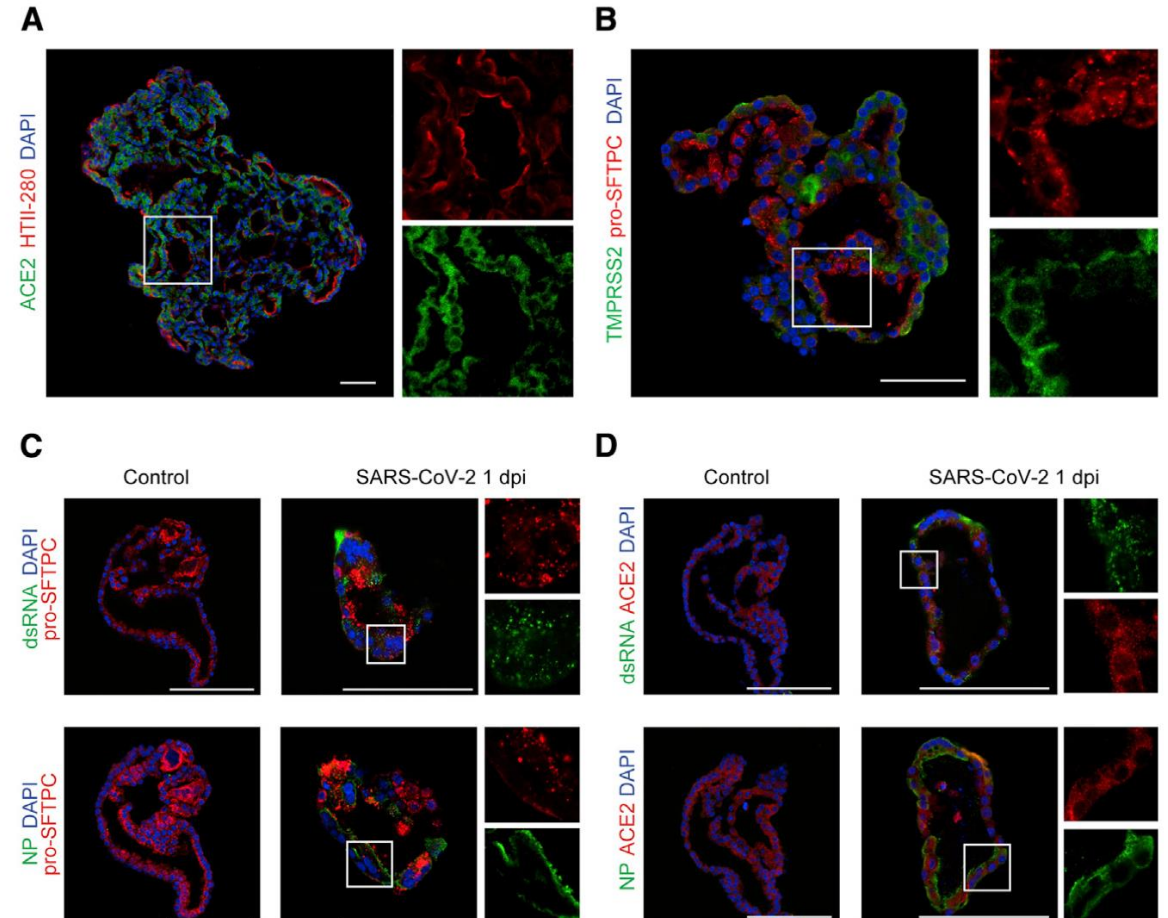
Correspondence

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In Brief

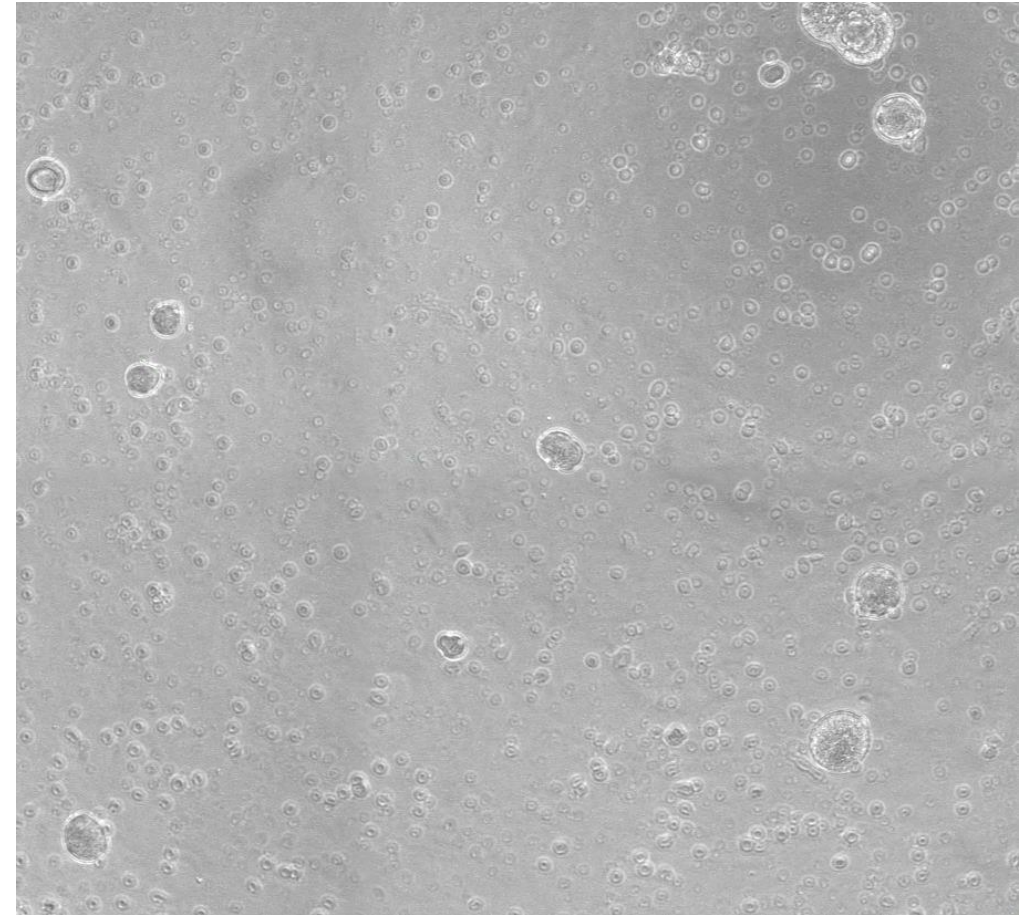
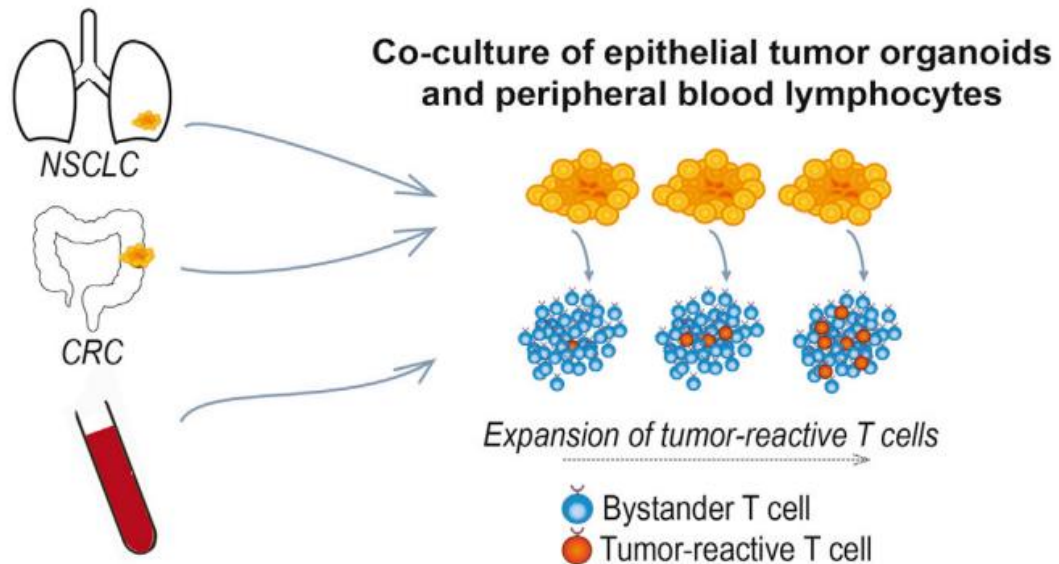
To study infection response to SARS-CoV-2, Youk et al. developed a 3D culture model of human alveolar stem cells in chemically defined conditions. They reveal that SARS-CoV-2 productively infects alveolar type 2 cells, resulting in an innate immune response. Full infection of individual cells was exhibited from a single viral entry.

Article



Lung cancer Organoids Co-cultured with Autologous Tumor-Reactive T Cells

**Generation of Tumor-Reactive T Cells
by Co-culture of Peripheral Blood Lymphocytes
and Tumor Organoids**



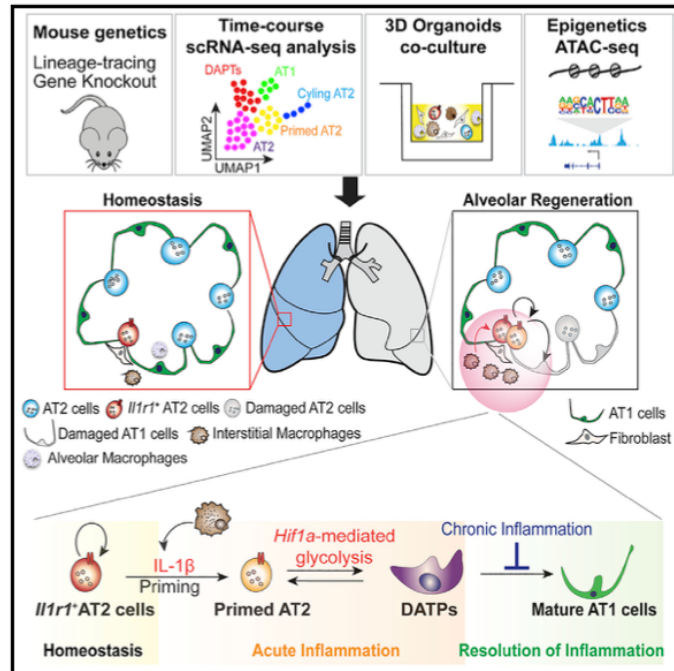
Identification of intermediate cells for Lung regeneration

Article

Cell Stem Cell

Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration

Graphical Abstract



Authors

Jinwook Choi, Jong-Eun Park,
Georgia Tsagkogeorga,
Motoko Yanagita, Bon-Kyoung Koo,
Namshik Han, Joo-Hyeon Lee

Correspondence

jhl62@cam.ac.uk

In Brief

Choi et al. show that inflammatory signals play a crucial role during alveolar regeneration after injury. They define stepwise differentiation trajectories for AT2 cells into AT1 cells via damage-associated transient progenitors (DAPTs) and find that unresolved inflammation stalls transition of DAPTs into mature AT1 cells.

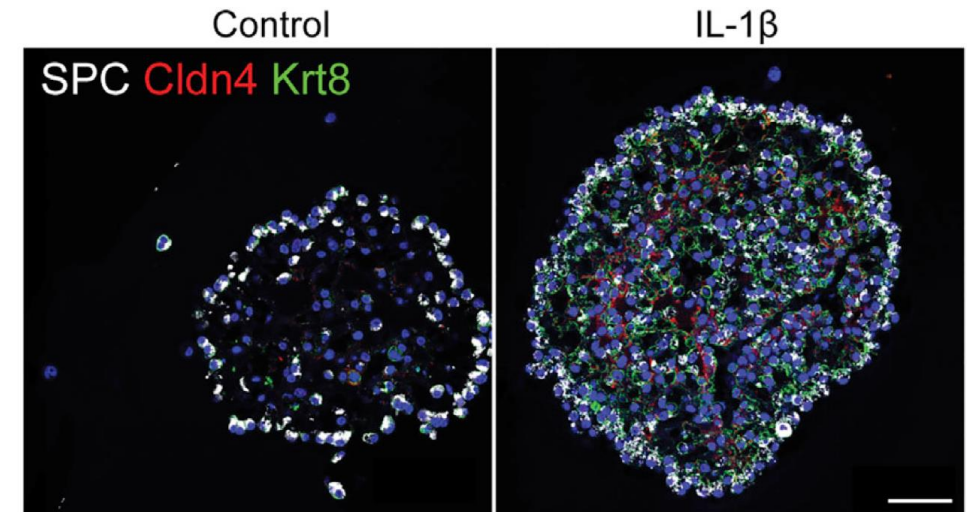
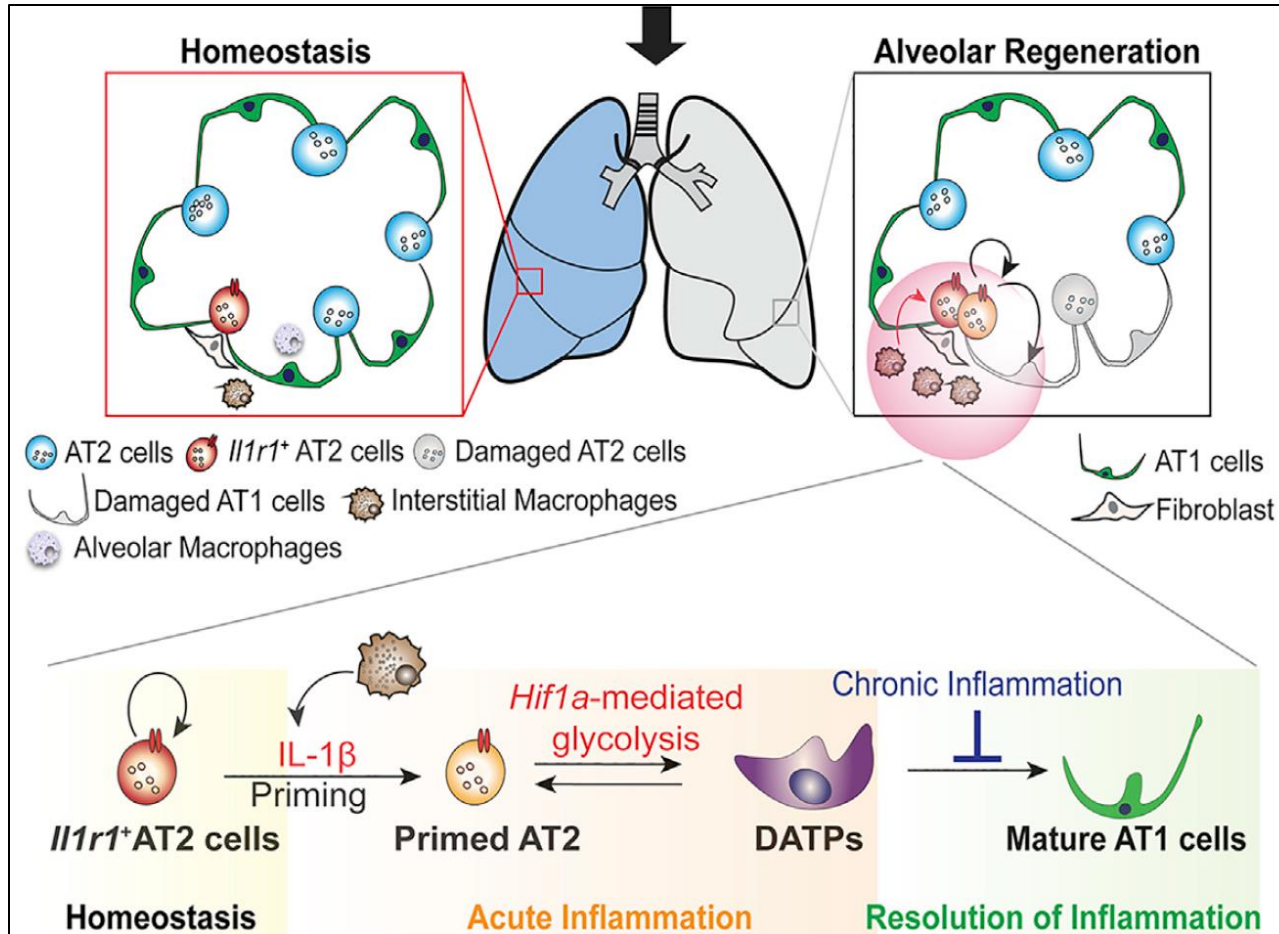


Joo-Hyeon Lee
Cambridge university

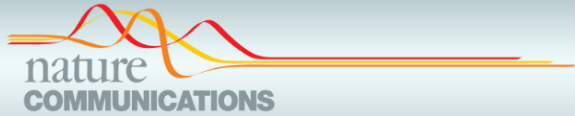


Jinwook Choi,
GIST

Damage-Associated Transition Progenitors



Lung cancer organoid Banking



ARTICLE

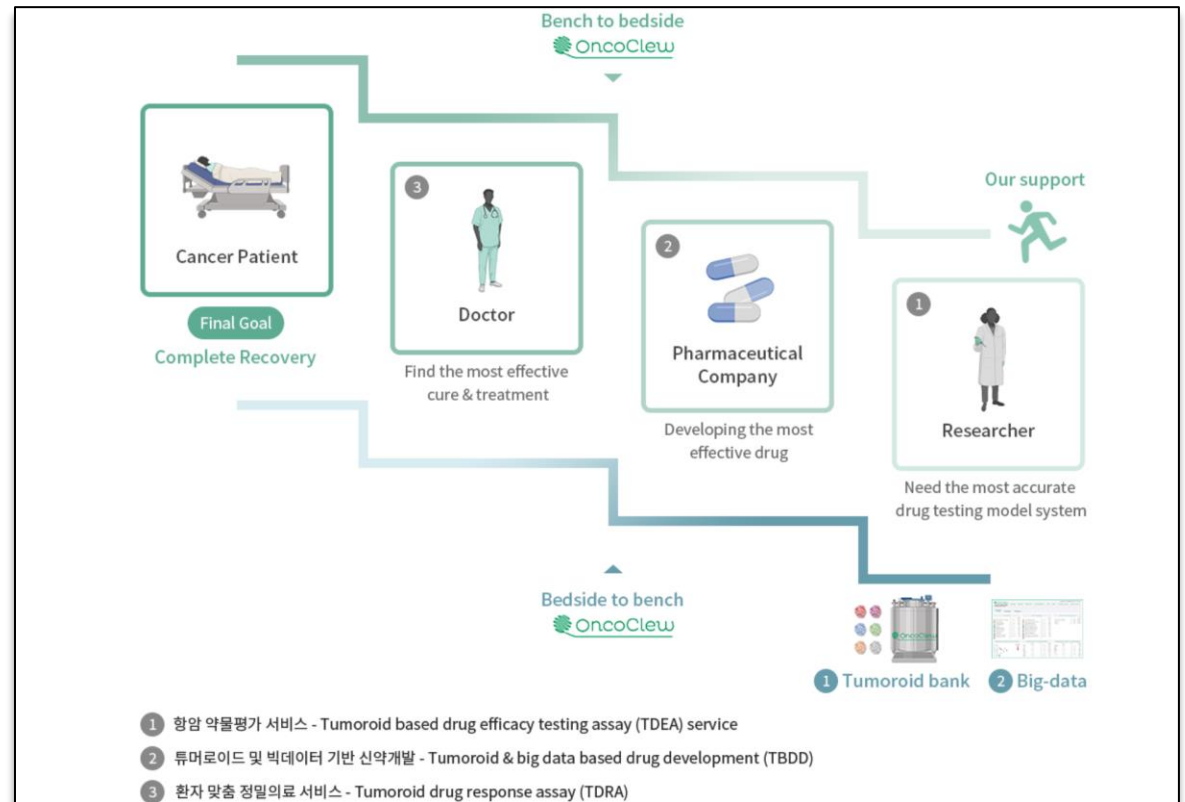
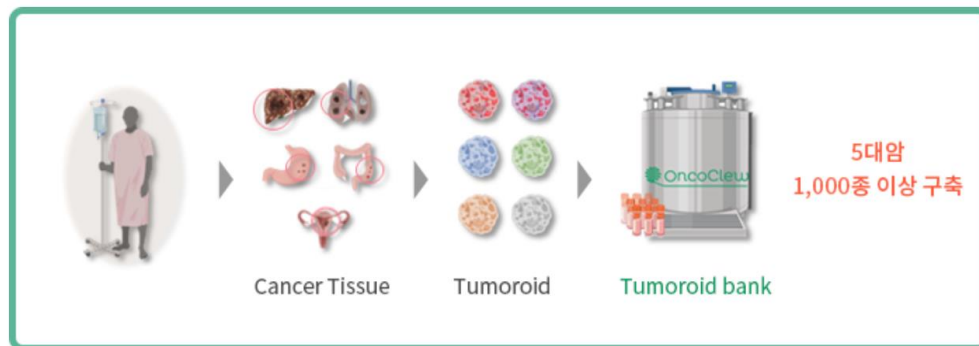
<https://doi.org/10.1038/s41467-019-11867-6>

OPEN

Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening

Minsuh Kim¹, Hyemin Mun¹, Chang Oak Sung^{1,2}, Eun Jeong Cho¹, Hye-Joon Jeon¹, Sung-Min Chun^{1,2}, Da Jung Jung³, Tae Hoon Shin³, Gi Seok Jeong³, Dong Kwan Kim⁴, Eun Kyung Choi⁵, Seong-Yun Jeong⁵, Alison M. Taylor⁶, Sejal Jain⁶, Matthew Meyerson⁶ & Se Jin Jang^{1,2}

온코클루 튜머로이드 바이오뱅크 (2022.4)



Precision medicine with Lung cancer organoid

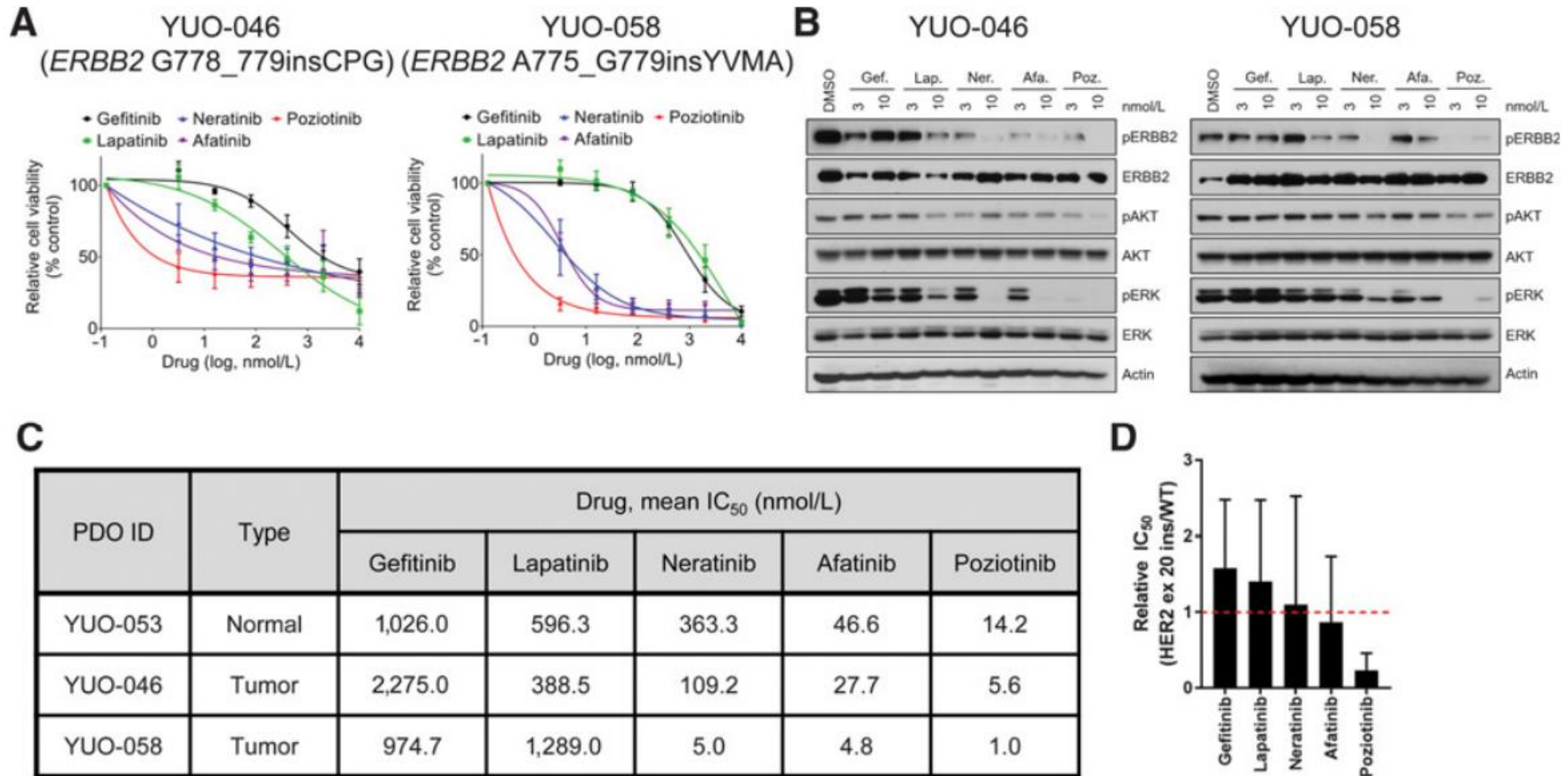
Modeling Clinical Responses to Targeted Therapies by Patient-Derived Organoids of Advanced Lung Adenocarcinoma

Seok-Young Kim¹, Sang-Min Kim², Sumin Lim³, Ji Yeon Lee³, Su-Jin Choi¹, San-Duk Yang¹, Mi Ran Yun¹, Chang Gon Kim¹, Seo Rin Gu³, Chaewon Park¹, A-Young Park¹, Sun Min Lim¹, Seong Gu Heo¹, HyunKi Kim², and Byoung Chul Cho⁴



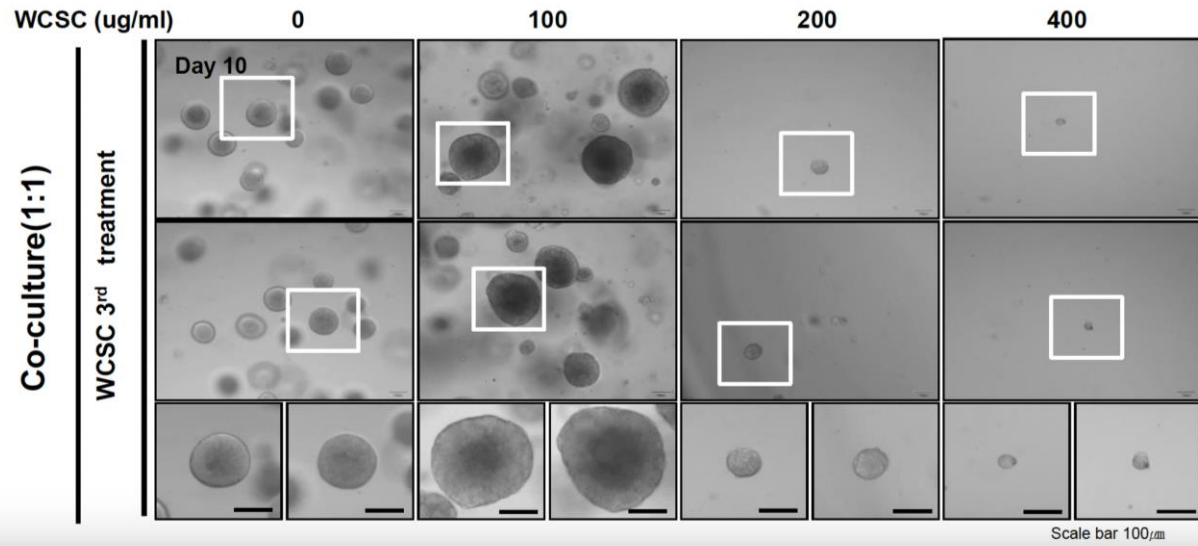
Malignant effusion, metastatic site specimen
--> lung cancer organoid

Lung cancer organoid harboring EGFR exon 20 insertion



The effects of smoking on the lung organoid

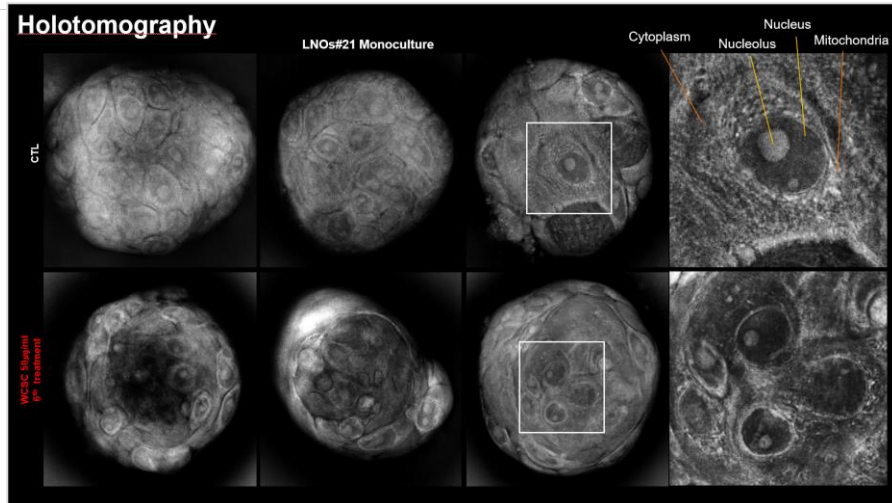
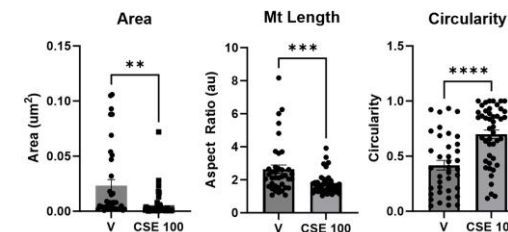
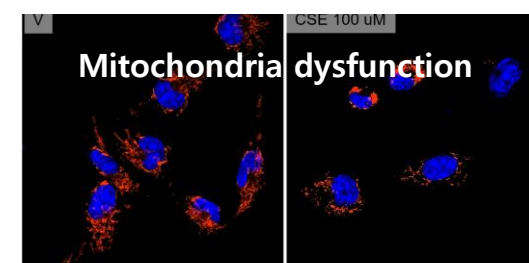
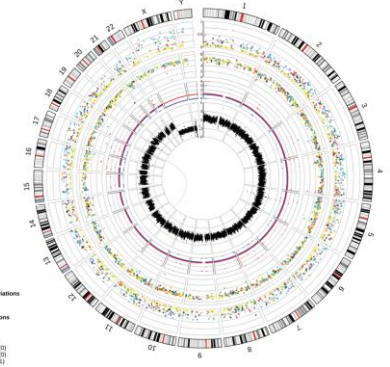
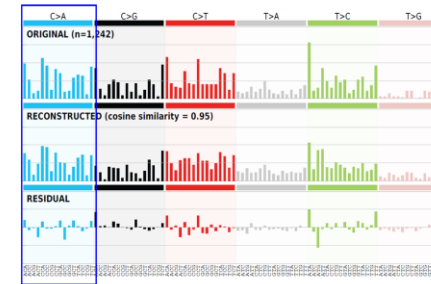
Whole cigarette smoke condensate (WCSC)



SBS4 is associated to direct DNA damage by tobacco smoke mutagens

Single Base Substitutions

| Signature | Estimated count | Contribution (%) | Proposed etiology |
|-----------|-----------------|------------------|--|
| SBS5 | 585.69 | 46.0 | Unknown (clock-like signature) |
| SBS3 | 266.3 | 20.9 | Defective homologous recombination DNA damage repair |
| SBS4 | 243.64 | 19.1 | Tobacco smoking |

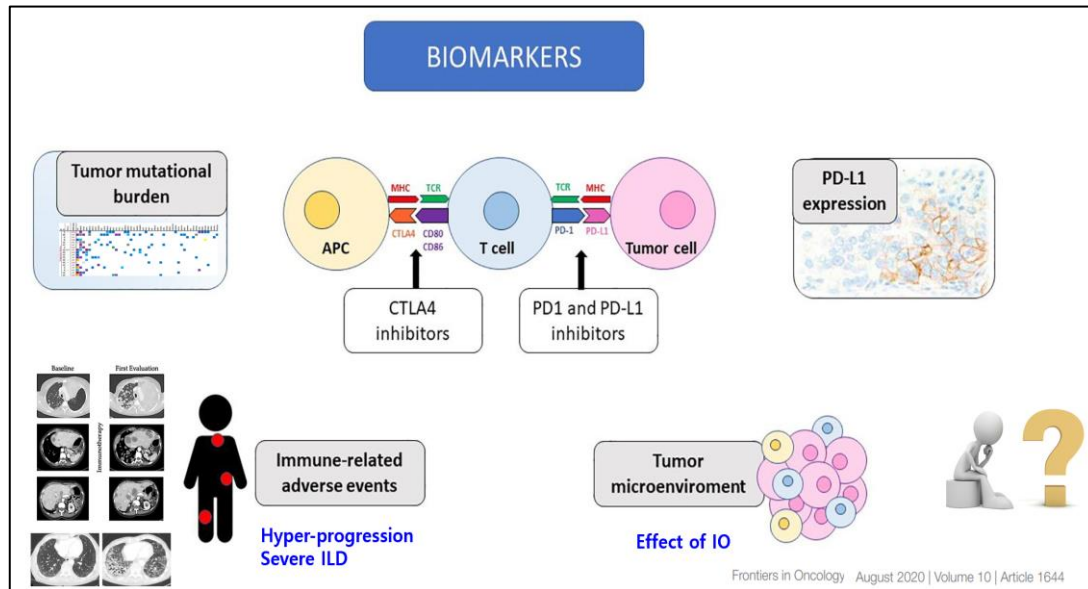


HOSPITAL



Challenging points in lung cancer diagnosis and treatment

1. **Biopsy is crucial but challenging** in lung cancer management.
2. Immunotherapy for cancer treatment: A double-edged sword: **Avoid severe adverse events**
3. Lack of Predictive Biomarkers for **best choice for this patient?**



"Which drug is the best choice for this patient?"

Target therapy

EGFR Exon 19 Deletion or Exon 21 L858R

- First-line therapy
 - ▶ Afatinib¹
 - ▶ Erlotinib²
 - ▶ Dacomitinib³
 - ▶ Gefitinib^{4,5}
 - ▶ Osimertinib⁶
 - ▶ Erlotinib + ramucirumab⁷
 - ▶ Erlotinib + bevacizumab^c (nonsquamous)⁸
- Subsequent therapy
 - ▶ Osimertinib⁹

EGFR S768I, L861Q, and/or G719X

- First-line therapy
 - ▶ Afatinib^{1,10}
 - ▶ Erlotinib²
 - ▶ Dacomitinib³
 - ▶ Gefitinib^{4,5}
 - ▶ Osimertinib^{6,11}
- Subsequent therapy
 - ▶ Osimertinib⁹

EGFR Exon 20 Insertion Mutation

- Subsequent therapy
 - ▶ Amivantamab-vmjw¹²
 - ▶ Mobocertinib¹³

KRAS G12C Mutation

- Subsequent therapy
 - ▶ Sotorasib¹⁴
 - ▶ Adagrasib¹⁵

ALK Rearrangement

- First-line therapy
 - ▶ Alectinib^{16,17}
 - ▶ Brigatinib¹⁸
 - ▶ Ceritinib¹⁹
 - ▶ Crizotinib^{16,20}
 - ▶ Lorlatinib²¹
- Subsequent therapy
 - ▶ Alectinib^{22,23}
 - ▶ Brigatinib^{22,23}
 - ▶ Ceritinib²⁴
 - ▶ Crizotinib²⁵
 - ▶ Lorlatinib²⁶

ROS1 Rearrangement

- First-line therapy
 - ▶ Ceritinib^{27,28}
 - ▶ Crizotinib²⁹
 - ▶ Entrectinib³⁰
- Subsequent therapy
 - ▶ Lorlatinib³¹
 - ▶ Entrectinib³⁰

BRAF V600E Mutation

- First-line therapy
 - ▶ Dabrafenib/trametinib³²
 - ▶ Dabrafenib³²
 - ▶ Vemurafenib
- Subsequent therapy
 - ▶ Dabrafenib/trametinib^{33,34}

NTRK1/2/3 Gene Fusion

- First-line/Subsequent therapy
 - ▶ Larotrectinib³⁵
 - ▶ Entrectinib³⁶

MET Exon 14 Skipping Mutation

- First-line therapy/Subsequent therapy
 - ▶ Capmatinib³⁷
 - ▶ Crizotinib³⁸
 - ▶ Tepotinib³⁹

RET Rearrangement


- First-line therapy/Subsequent therapy
 - ▶ Selpercatinib⁴⁰
 - ▶ Pralsetinib⁴¹
 - ▶ Cabozantinib^{42,43}

ERBB2 (HER2) Mutation

- Subsequent therapy
 - ▶ Fam-trastuzumab deruxtecan-nxki⁴⁴
 - ▶ Ado-trastuzumab emtansine⁴⁵

PD-L1 ≥50% First-line Therapy

PD-L1 ≥1-49% First-line Therapy



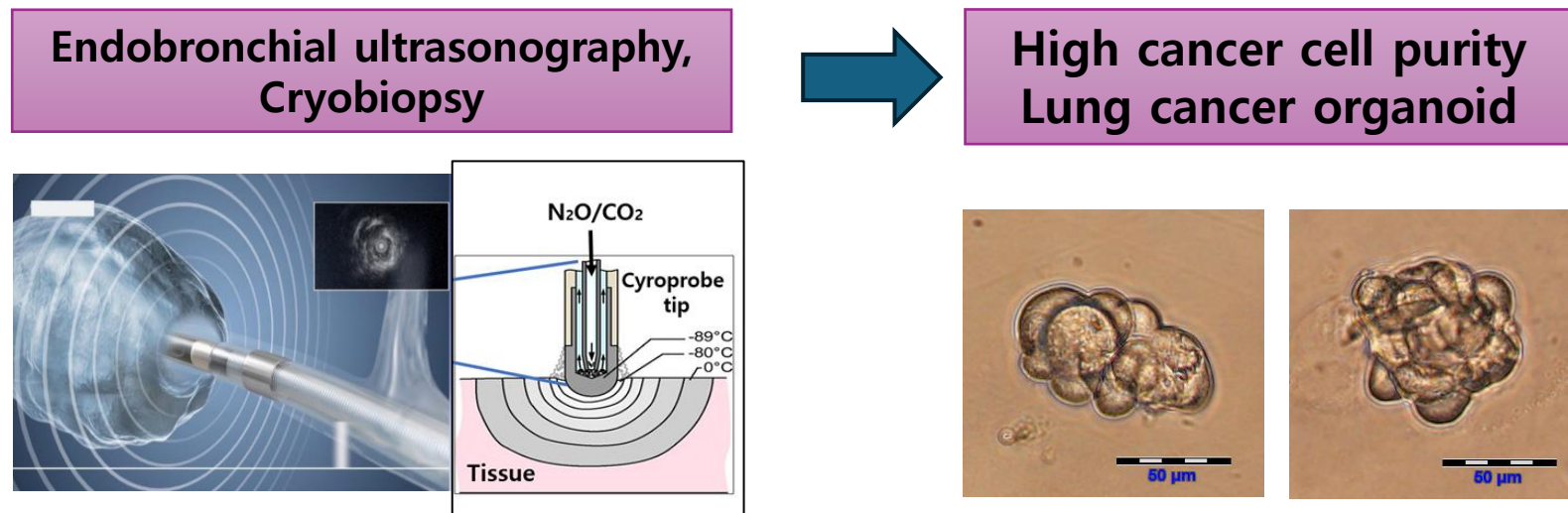
Challenging points in lung cancer diagnosis and treatment


1. Immunotherapy for cancer treatment: A double-edged sword: **Avoid severe adverse events**
2. Lack of Predictive Biomarkers for **Best Drug for this patient?**



Challenging points in lung cancer organoid

1. To create personalized medicine, it is necessary to develop a **lung cancer organoid using tissue from a biopsy.**
2. It is **very difficult** to make a lung cancer organoid with a conventional biopsy tissue (especially with peripheral lung cancer).
3. It is **challenging to predict the proportion of cancer cells** within a lung cancer organoid.



- 
- An aerial view of a large, modern hospital complex. The main building is a multi-story structure with a light-colored facade and a grid of windows. A prominent sign on the top of the building reads "경남대학교병원" (Gyeongsang National University Hospital). The hospital is surrounded by a landscaped courtyard with greenery and a curved road. Several cars are parked in the foreground, and a few people can be seen walking around the area.
1. Surgery tissue is available only for early lung cancer patients.
 2. Success rate of organoid culture derived from biopsy sample is low.

Overcome the big hurdle of lung cancer organoid

비수술 조직 생검을 이용한 폐암 오가노이드

비소세포 폐암

| | | | |
|---------------|--------------|---------------|---------------|
| 1기 (29.7%) | 2기 (8.4%) | 3기 (18.2%) | 4기 (43.7%) |
| 수술 가능 | | | |

소세포폐암

| | |
|----------------|----------------|
| 제한성 (29.7%) | 확장성 (70.3%) |
| 수술 | |

비수술적 조직 생검 (원발 부위)

종격동/경부 림파선 조직 생검

전이병변 조직 생검, 악성 흉수 세포

폐암 재발, 진행시 재조직 검사(re-biopsy)

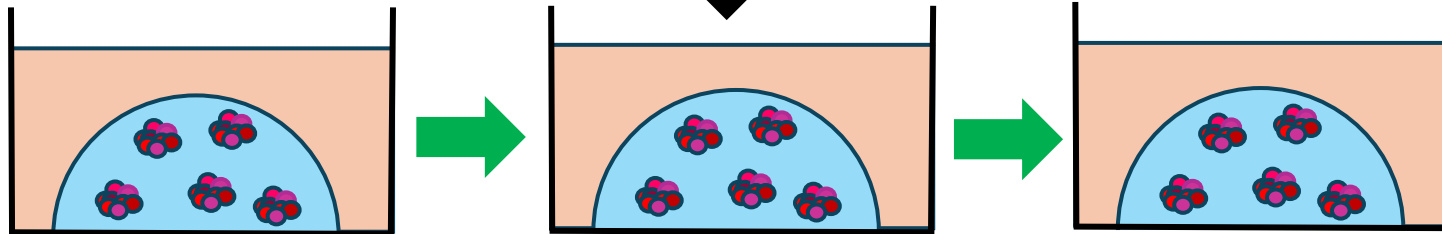
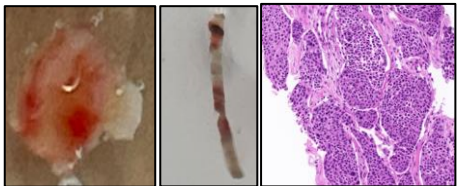
비수술적 조직 생검

림파선
조직생검

전이, 악성 흉수,
림파선 생검

재조직 검사

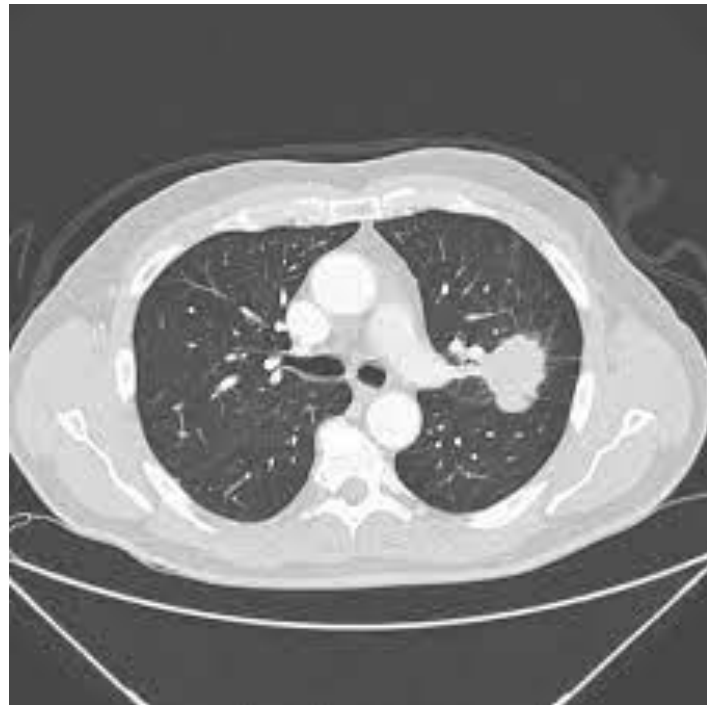
비수술(생검)조직 유래
폐암 오가노이드



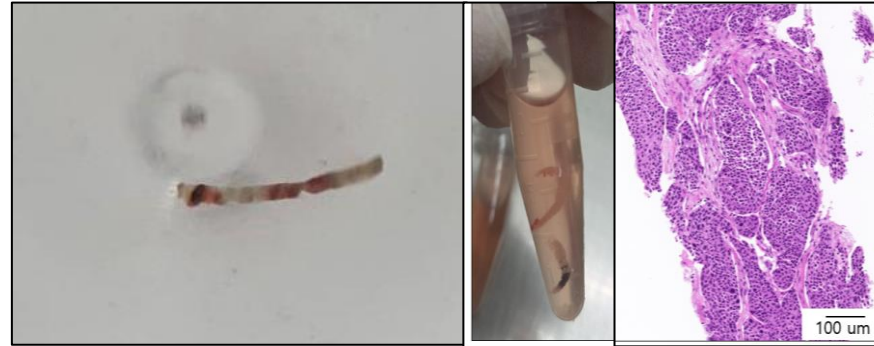
고순도 폐암 오가노이드
(High purity lung cancer organoid)

Drug screening
in 2~3 weeks for
personalized medicine

Lung cancer organoid derived from small biopsy tissue



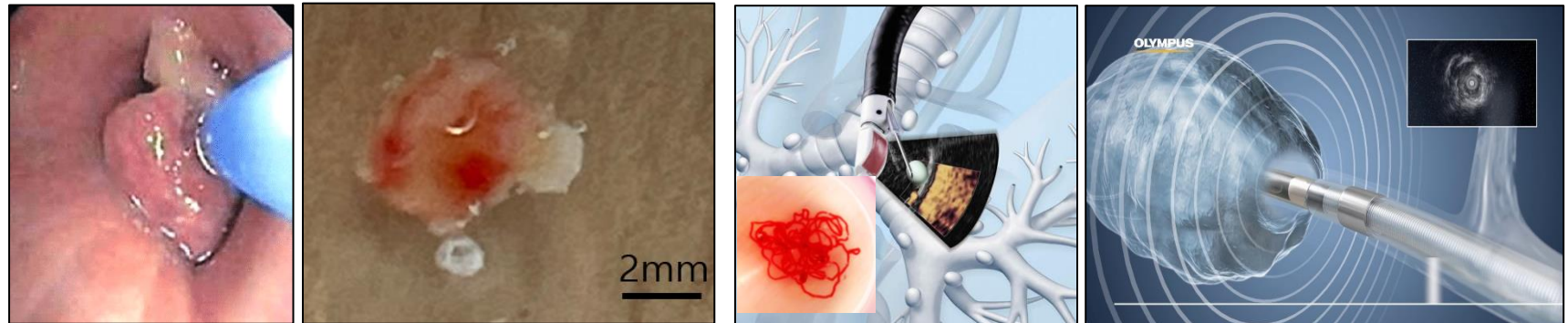
(Percutaneous needle biopsy)



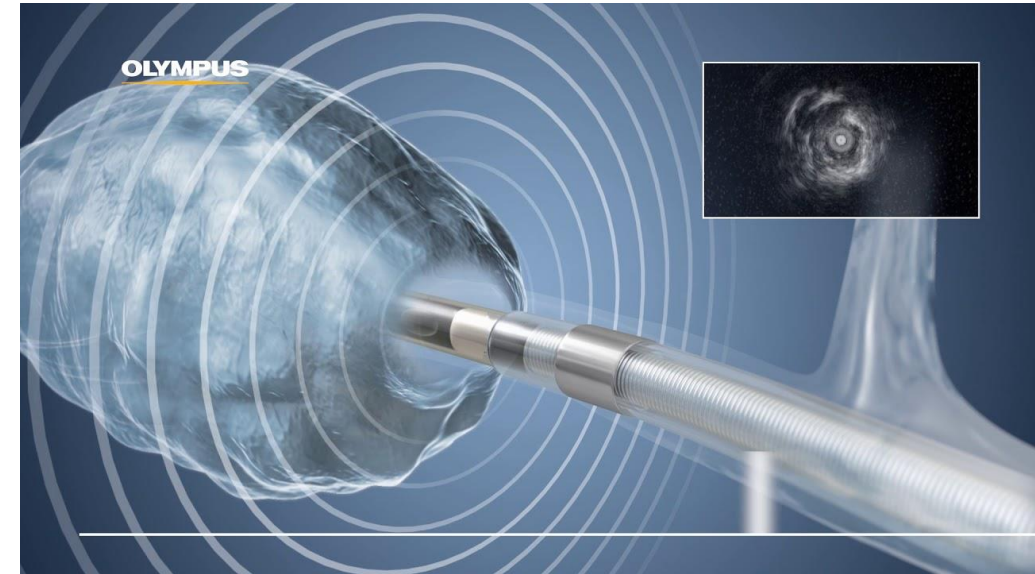
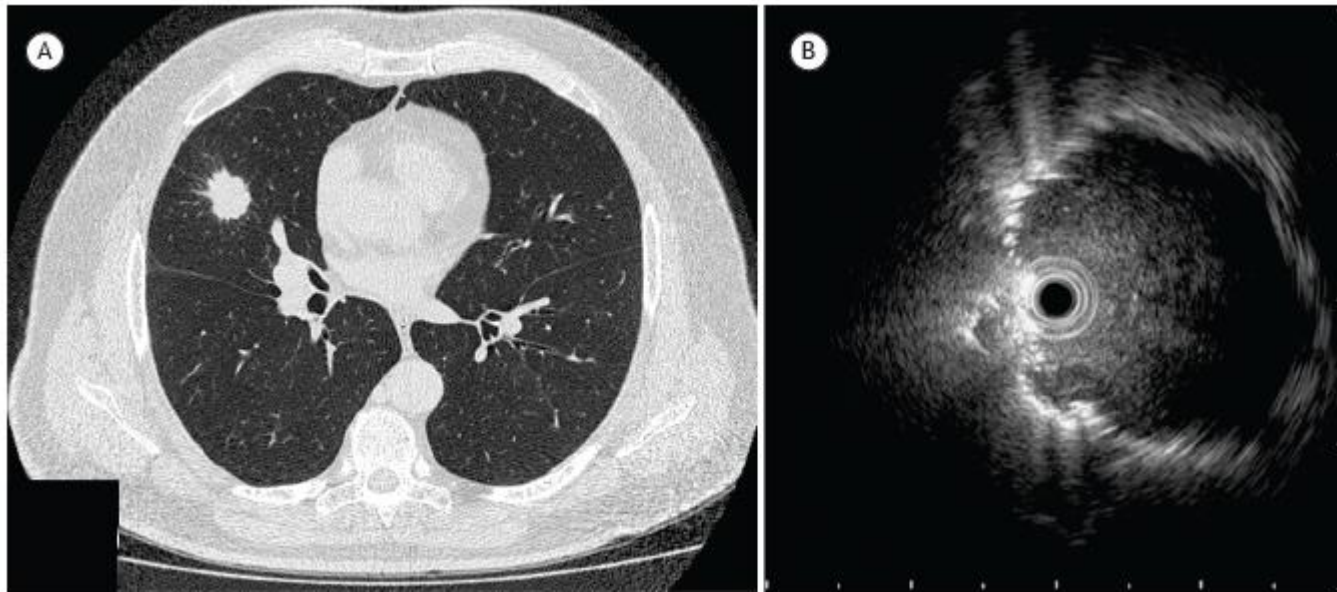
(Bronchoscopic biopsy)



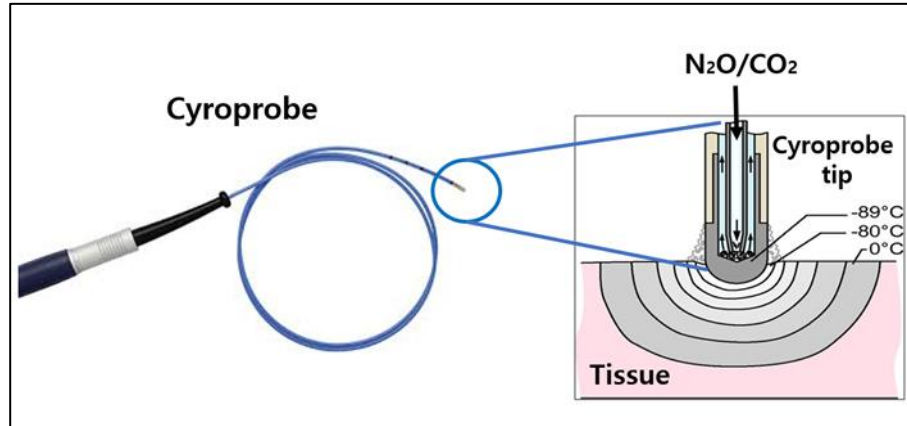
(Linear, Radial EBUS)



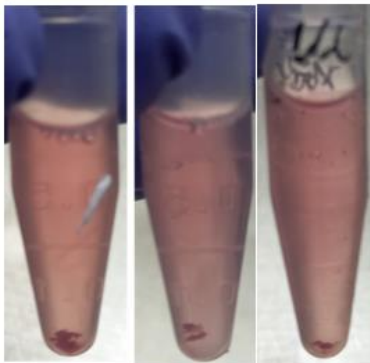
Radial EBUS



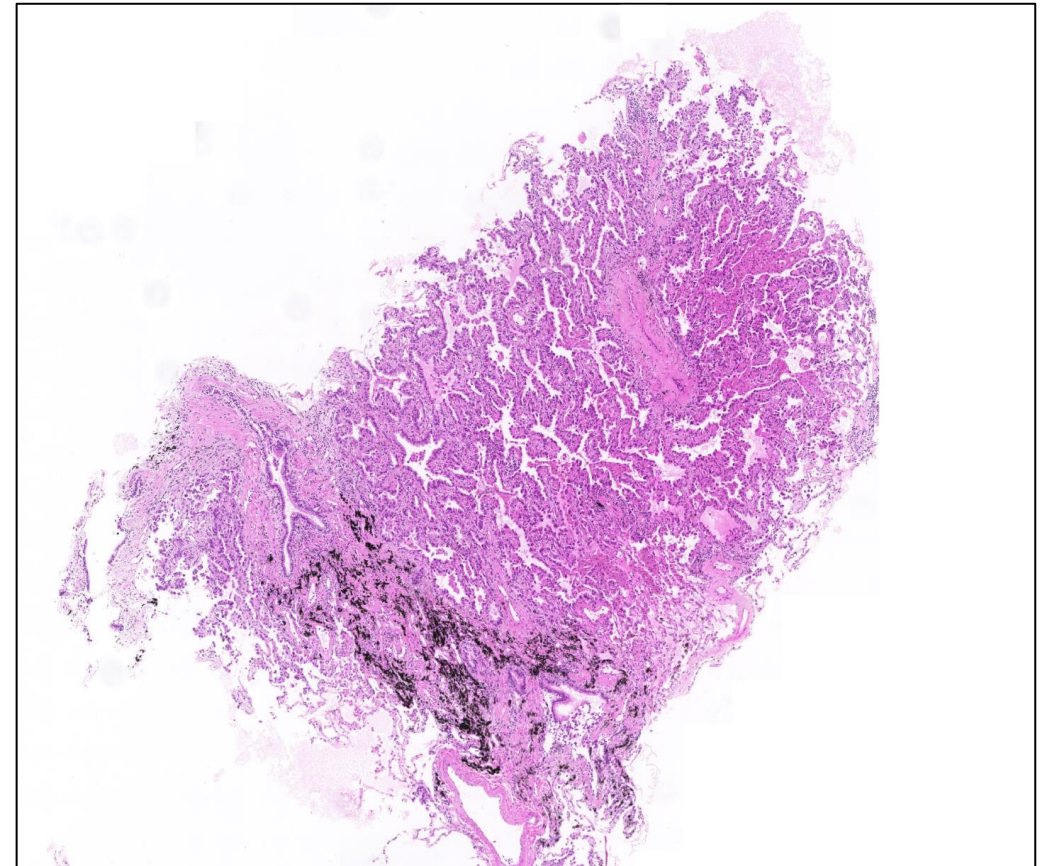
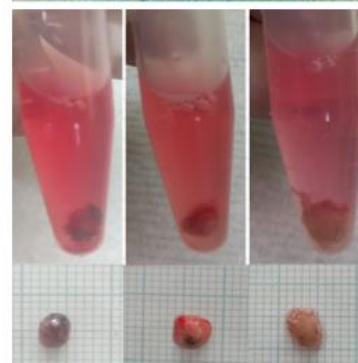
Lung cancer organoid derived from cryobiopsy samples



Forceps biopsy

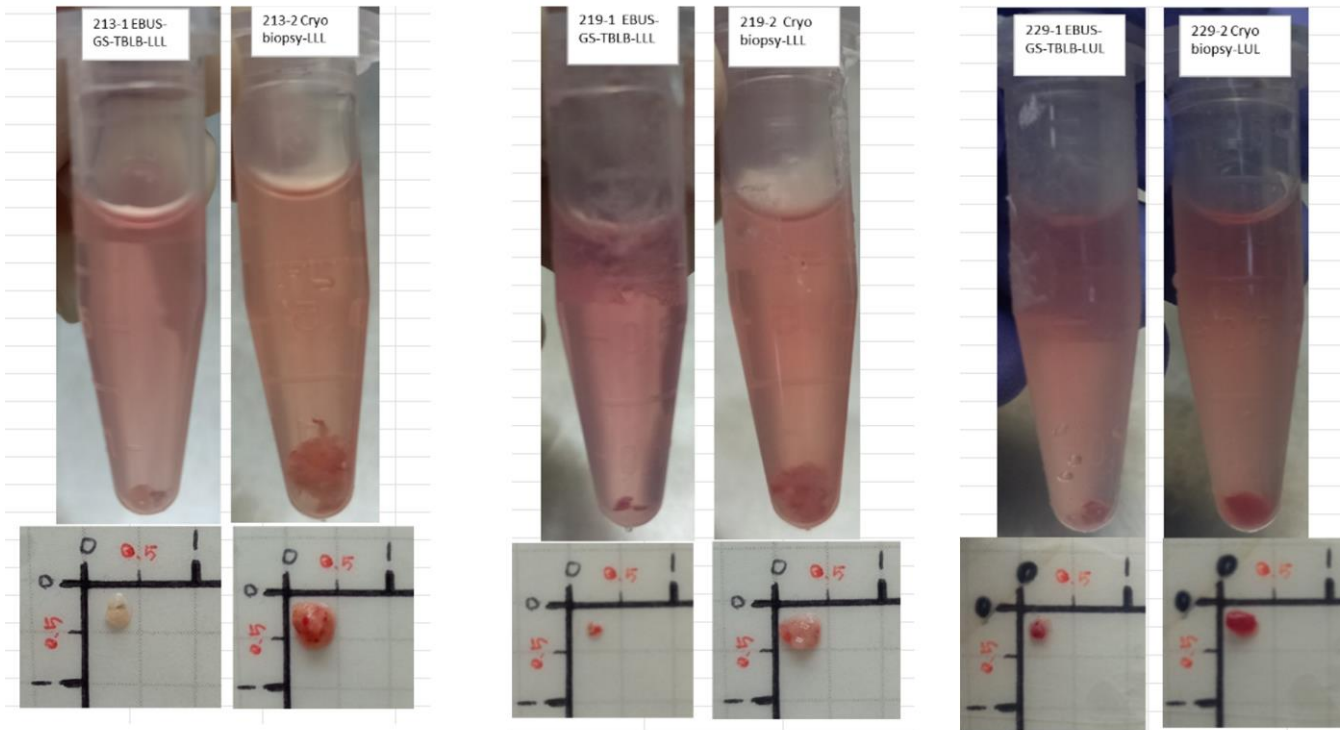


Cryobiopsy



High purity of lung cancer cell

Forcep biopsy와 Cryobiopsy 비교



| | | 급속동결-해동법 | 일반 겸자 생검 |
|---|------|-------------|-------------|
| 1 | 66/남 | 오가노이드 배양 성공 | 오가노이드 배양 실패 |
| 2 | 68/남 | 오가노이드 배양 성공 | 오가노이드 배양 실패 |
| 3 | 84/여 | 오가노이드 배양 실패 | 오가노이드 배양 실패 |
| 4 | 61/여 | 오가노이드 배양 성공 | 오가노이드 배양 실패 |
| 5 | 44/여 | 오가노이드 배양 성공 | 오가노이드 배양 실패 |
| 6 | 58/남 | 오가노이드 배양 성공 | 오가노이드 배양 성공 |

The more organs, the more stem cells for organoid

Usual organoid forming efficiency is 1/100~1/1000

Human lung organoid culture protocol

natureprotocols

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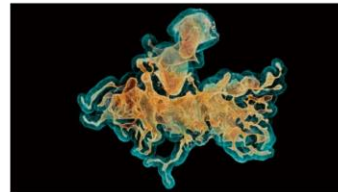
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[nature](#) > [nature protocols](#) > collection

Collection 27 February 2023

Organoid protocols

Organoid culture systems are self-renewing 3D models derived from stem cells (pluripotent or adult-derived) or cancer cells that recapitulate characteristics of their tissues of origin. Here, we provide protocols for growing and studying various types of organoid (brain, skin, kidney, liver, bile duct, prostate, placenta, breast, colon, stomach, pancreas, lung, heart, and blood vessel) generated from both healthy and cancer cells.



Editors

[Mel Clyne](#) & [Andrew Economou](#)

Protocols

Protocol

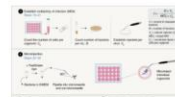
11 Aug 2021

[Nature Protocols](#)

Intestinal organoid cocultures with microbes

This protocol comprises various methods to coculture organoids (particularly human small intestinal and colon organoids) with microbes, including microinjection into the lumen and periphery of 3D organoids and exposure of organoids to microbes in a 2D layer.

Jens Puschhof, Cayetano Pleguezuelos-Manzano ... Hans Clevers



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Human Lung Organoid Culture Protocol

| |
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| Protocols |
| Apoptosis Assays |
| Enzyme Assays |
| Flow Cytometry |
| ICC/IHC Protocols |
| Immunocytochemistry (ICC) Protocols |
| Immunohistochemistry (IHC) Protocols |
| Immunology |
| Luminex |
| Neural Cell Culturing |

This protocol outlines a method for culturing normal human lung organoids based on protocols described by Norman Sachs and Hans Clevers (1). The procedure that is described allows for the long-term expansion of airway organoids *in vitro* from adult human lung tissue. Starting with a suspension of isolated and dissociated human lung tissue, the reagents and steps necessary to generate and passage human lung organoids are fully detailed, allowing for the generation of an *in vitro* lung model that can be used for pulmonary disease modeling and drug toxicity studies. This lung organoid protocol utilizes reagents across the Bio-Techne portfolio including recombinant proteins, small molecules, media supplements, and Cultrex® Organoid Qualified Basement Membrane Extract (BME) as a scaffold.

The protocol provides some specific tips and recommendations for culturing human lung organoids including:

- Recommended media for expanding and differentiating lung organoids
- Considerations and tips for starting lung organoid cultures
- Methods to optimize Cultrex Organoid Qualified BME as a matrix scaffold
- Tips for successful passaging and cryopreservation



[Download Human Lung Organoid Culture](#)

Cell isolation → single cell로 만들기

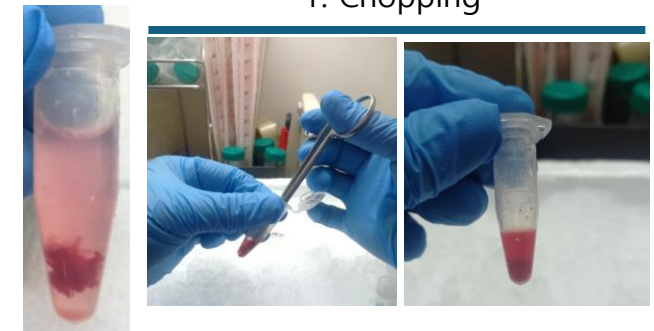
1. Chopping & Enzyme digestion : Tissue fragments를 Cold PBS로 washing해준 후 최대한 chopping하고, DMEM/F12를 넣고 37°C에서 2hr동안 반응시킨다.

(enzyme digestion을 생략하고 homogenizer를 포함한 mechanical digestion만 하기도 함)

2. Pipetting으로 여러 번 섞어 주고, 70 μ m cell strainer로 걸러준다.

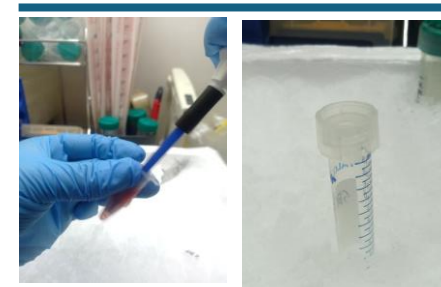


1. Chopping



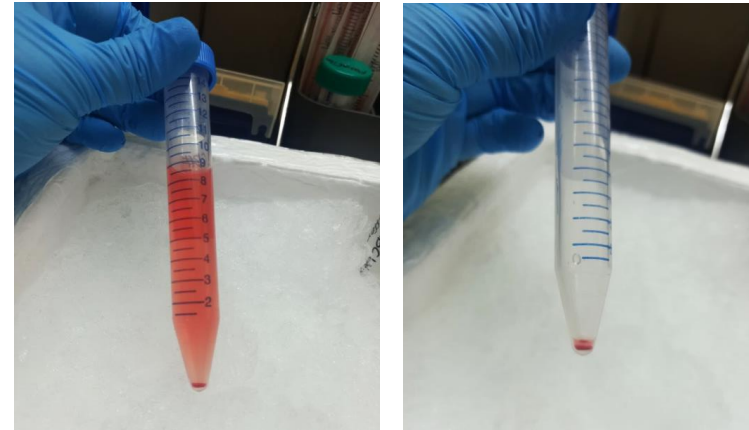
Homogenizer

2. 70um strainer filter

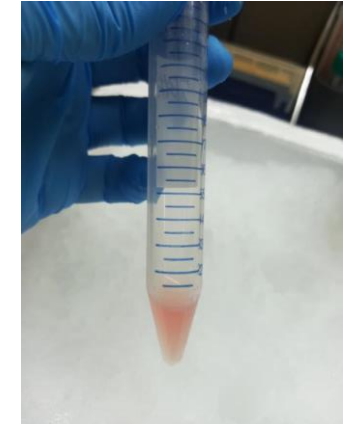


Cell isolation

3. Centrifuge → pellets



4. RBC lysis

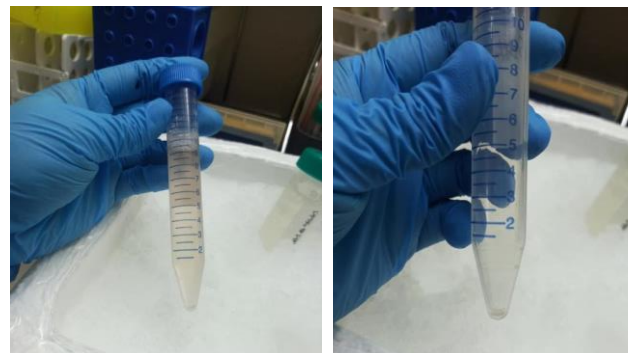


3. Centrifuged at $112 \times g$ for 3min

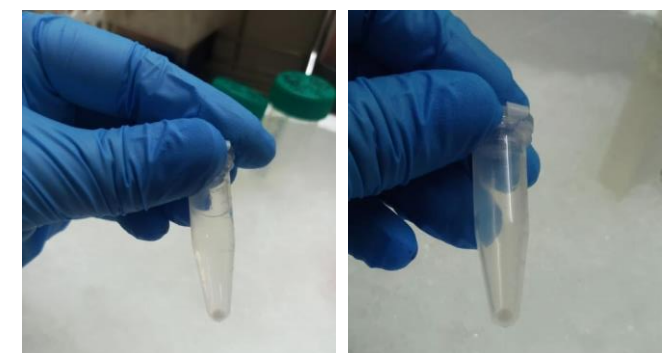
4. 상등액은 버리고 RBC lysis → centrifuge

5. Cell pellets washing → centrifuge

Centrifuge → pellets



5. Washing



Cell isolation

6. Pallet 에 100ul 의 Growth medium DMEM/F12 을 넣고 suspension.

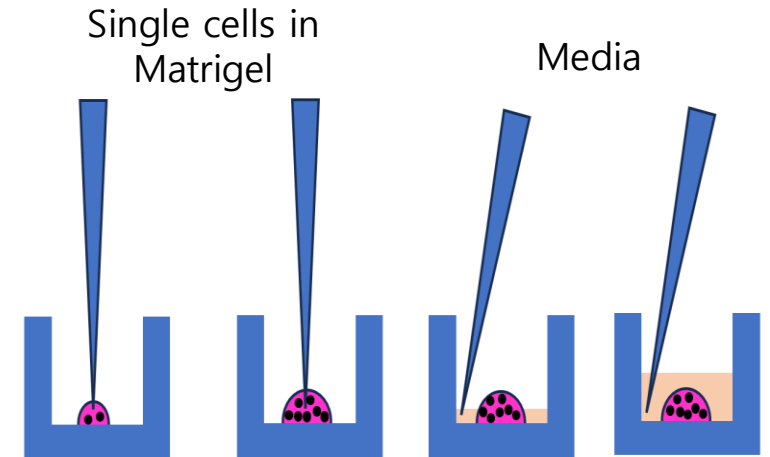
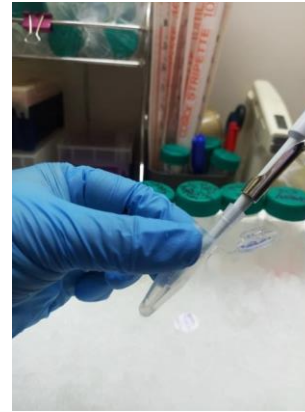
7. 50~100ul cell을 100ul Matrigel (Corning, 354230)을 넣고 (Cell : Matrigel = 1 : 1~2 비율) bubble이 생기지 않게 섞어준다.

8. Plate 뚜껑을 닫고 뒤집어서 37°C incubator에 15~30분 정도 incubation 시키면서 천천히 dome 형태로 굳힌다.

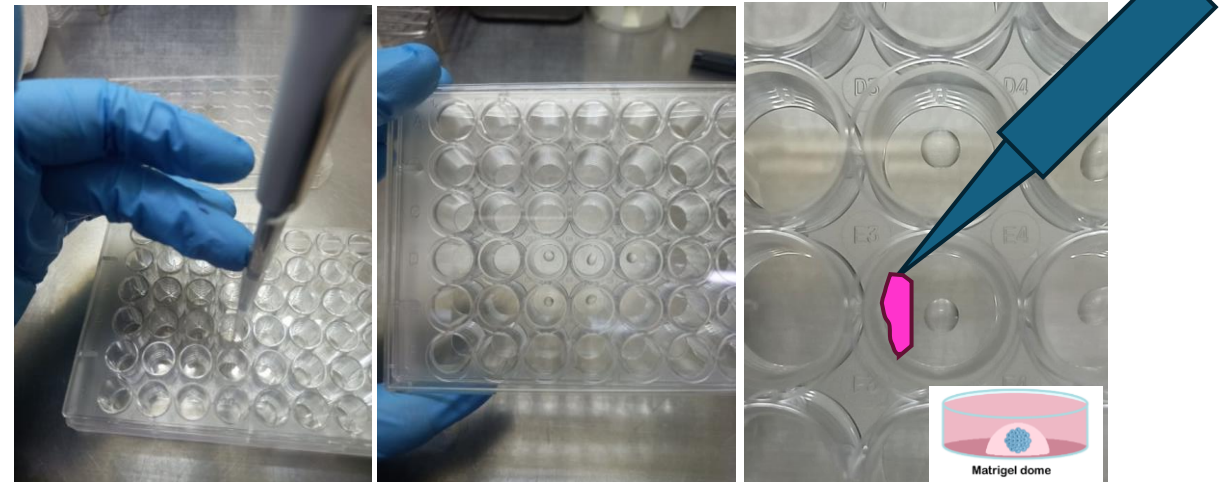
9. 15~30분 후 꺼내서 plate를 정방향으로 뒤집은 후, 300ul media을 Matrigel에 닿지 않게 well side 쪽으로 넣어준다.

7. 배지는 3~4일에 한번씩 바꿔주며, passage는 1~3주 후에 해준다. 오가노이드마다 자라는 속도가 다르기 때문에 중간중간 현미경으로 확인 후 passage해준다.

6. Cell suspension

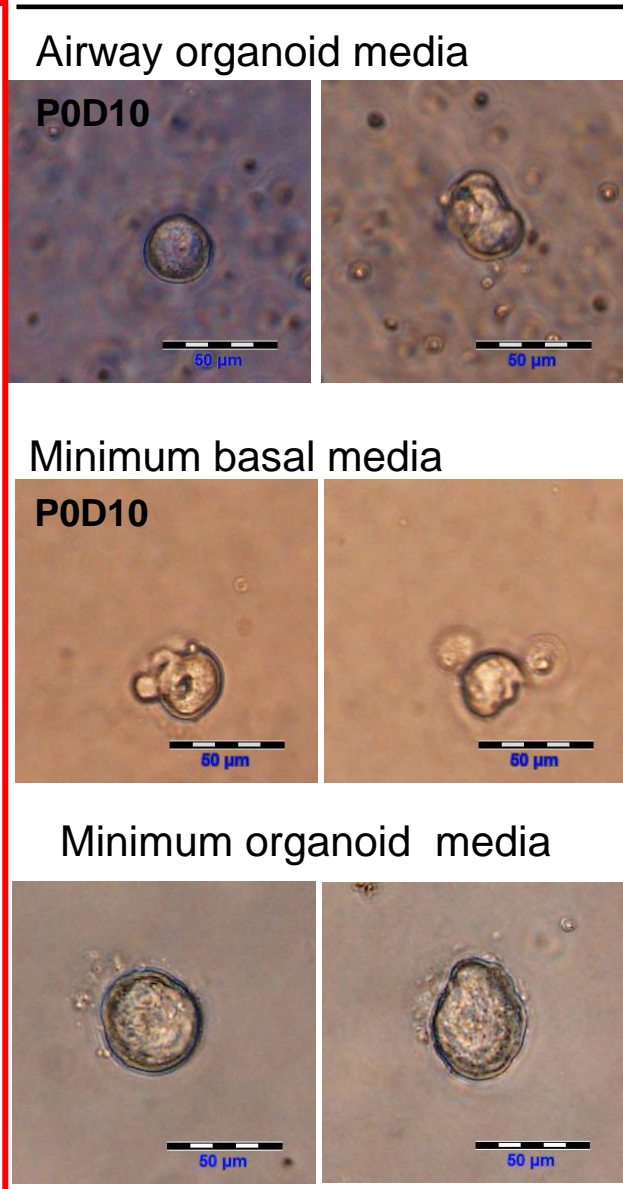
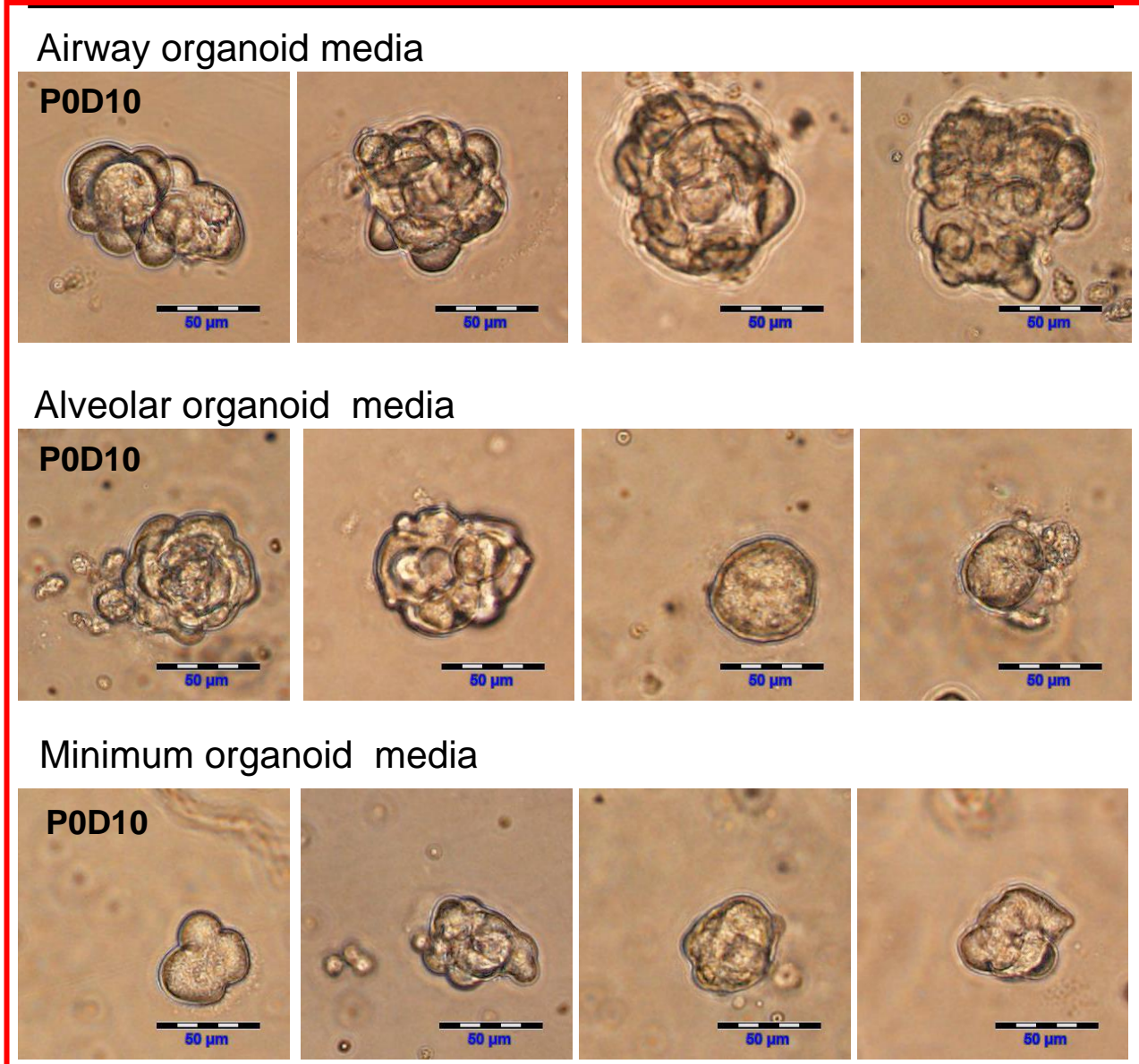


7. Seeding (cell embedding in Matrigel) in 48 well plate



Enzyme free CNUH method

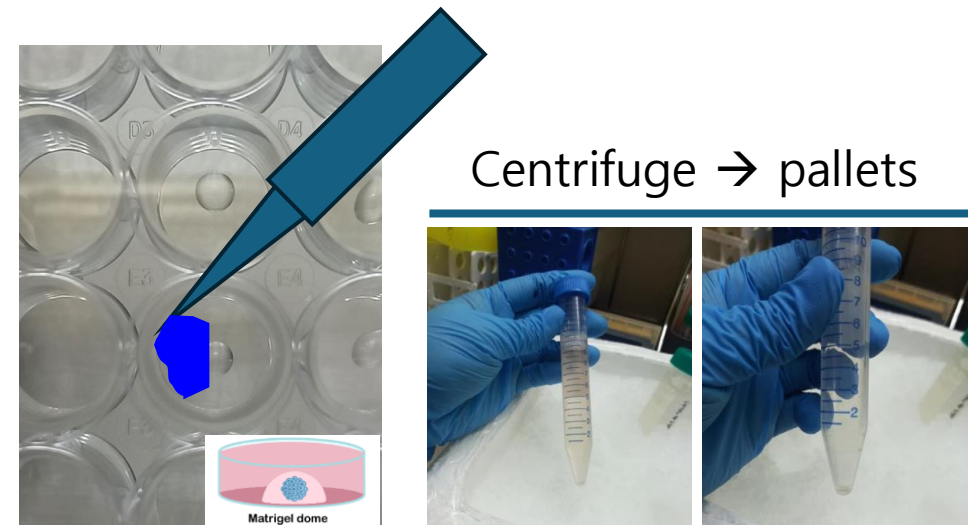
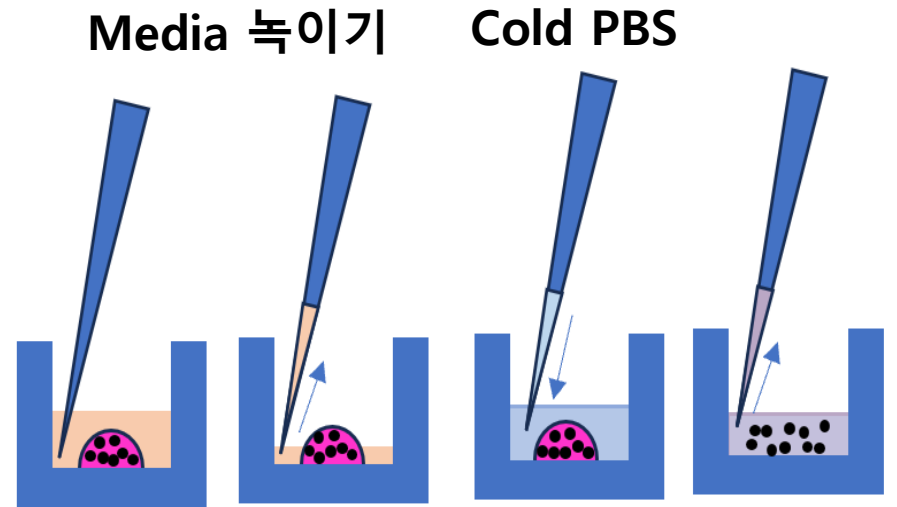
Enzyme method



Scale bar 50μm

Passaging

1. **Matrigel 녹이기**: media를 제거하고 **Cold PBS**를 굳어 있는 Matrigel에 떨어트린 후, pre-cooled pipette으로 모은다.
2. Centrifuged at 112 x g for 3min at 4°C.
3. Sup.은 버리고, cell pallet에 2ml TrypLE Express (Thermo fixer; 12304013)을 넣고, 37°C에서 10min 동안 incubation.
4. 10ml DMEM/F12 (containing 10% FBS)을 넣고 washing한다
5. Centrifuged at 112 x g for 3min at 4°C.
6. Sup.은 버리고, cell pallet +Media : Matrigel = 1 : 1~3 으로 resuspension.
7. 새로운 plate에 seeding 해준다.



Cryopreservation of organoid

1. **Cold PBS**를 굳어 있는 Matrigel에 떨어트린 후, pre-cooled pipette으로 모은다.
2. Centrifuged at 112 x g for 3min at 4°C.
3. 한번 더 washing 해준 후, Centrifuged at 112 x g for 3min at 4°C.
4. **Freezing media : culture media 7 : ES grade FBS (Gibco) 2 : DMSO (sigma) 1**에 cell pellets suspension해서 Cryo-vial에 넣는다.
5. Cryo-vial을 freezing container에 넣고 deep freezer → 액체 질소 보관

천천히 얼리고.



DMSO: 동결보존제



Cryo vial



Freezing Container

Thawing of cryopreserved organoid

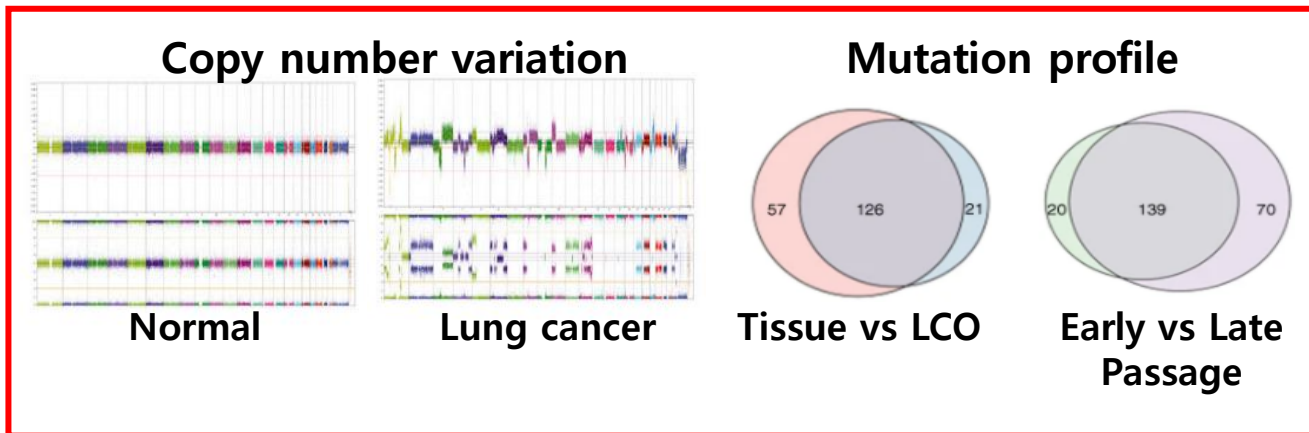
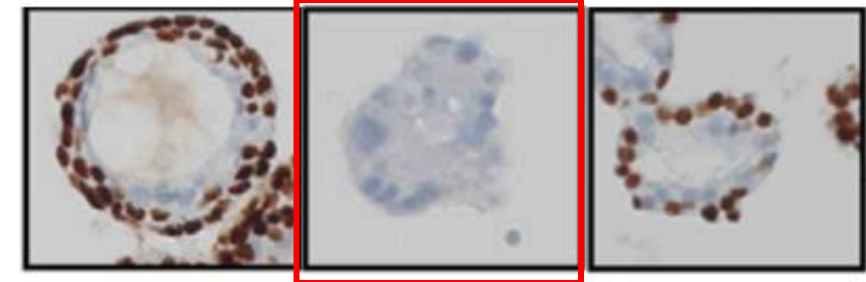
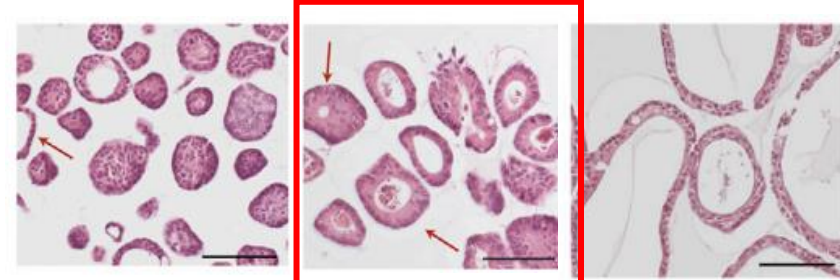
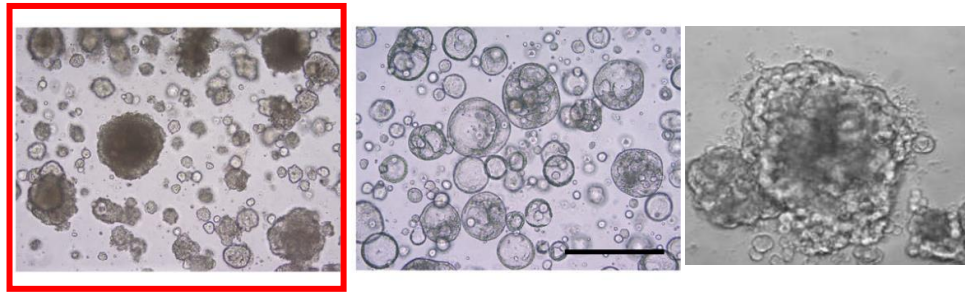
1. Cryo-vial을 37°C water에 녹여준다.
2. 10ml medium으로 pellet을 suspension
3. Sup.은 버리고, seeding method step을 진행한다.

빠르게 녹이고.



Validation of lung cancer organoid

- Morphology, histology, Immunohistochemistry (p63 expression, polarity)
- Copy number variation, Variant allele frequency, Mutation profile, Gene expression

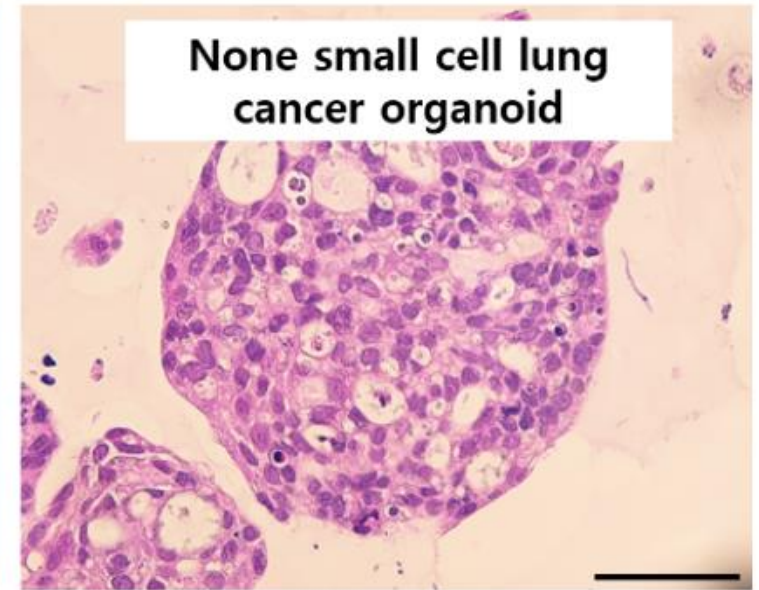
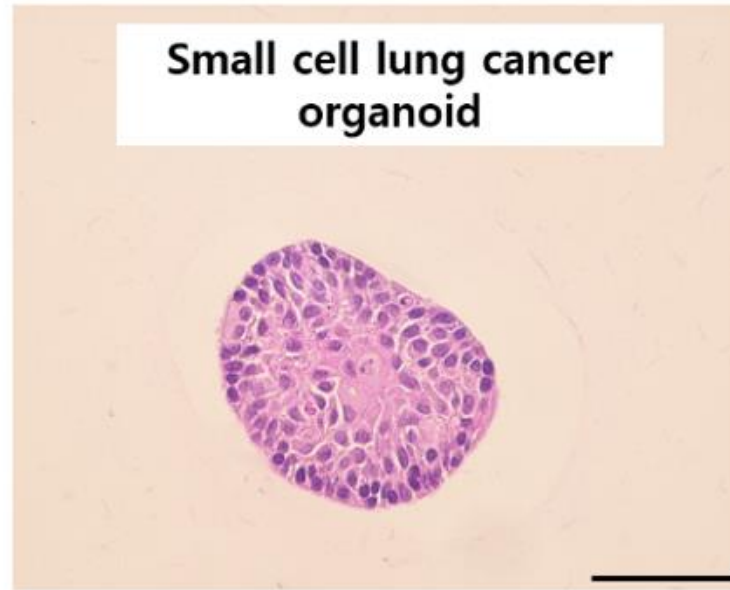
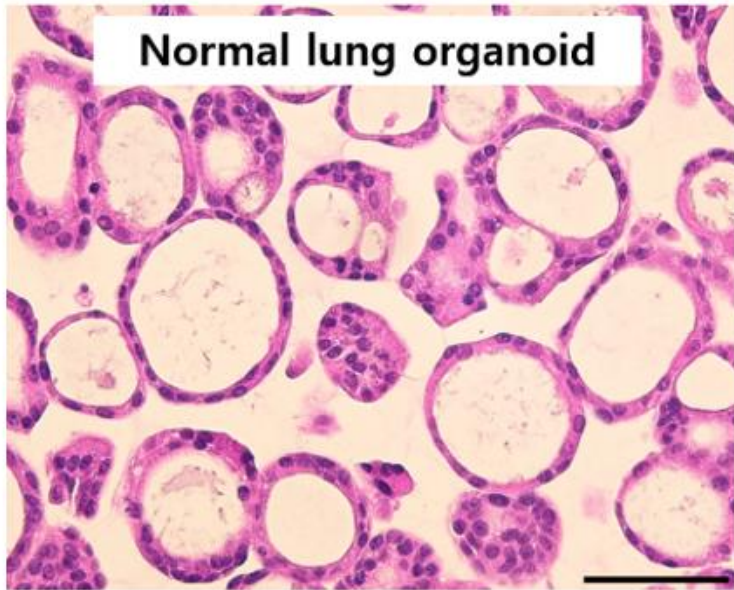




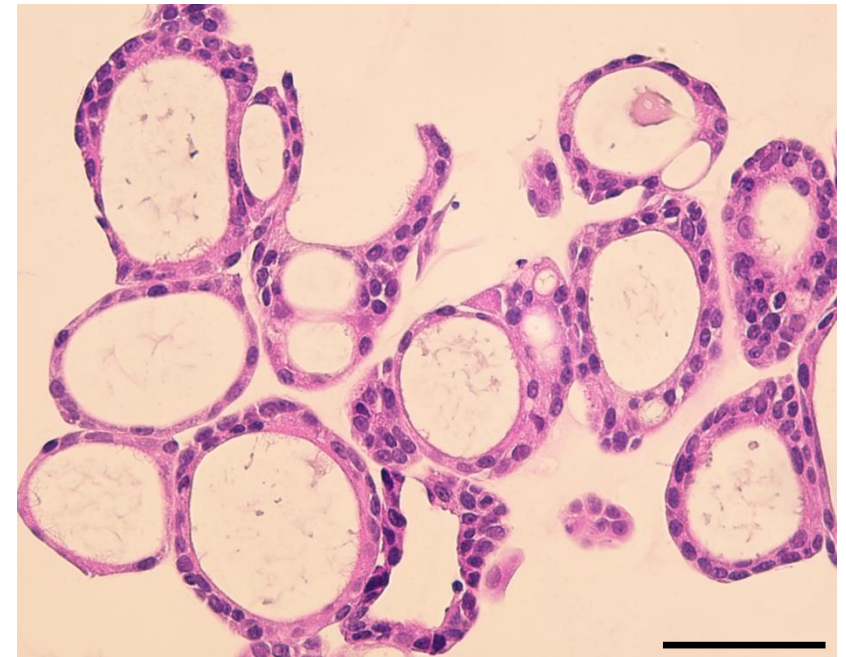
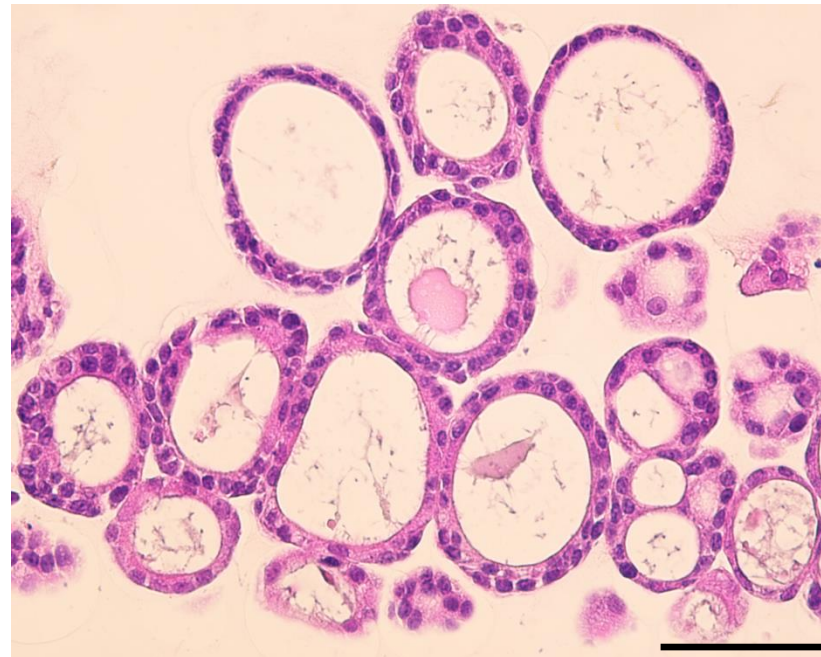
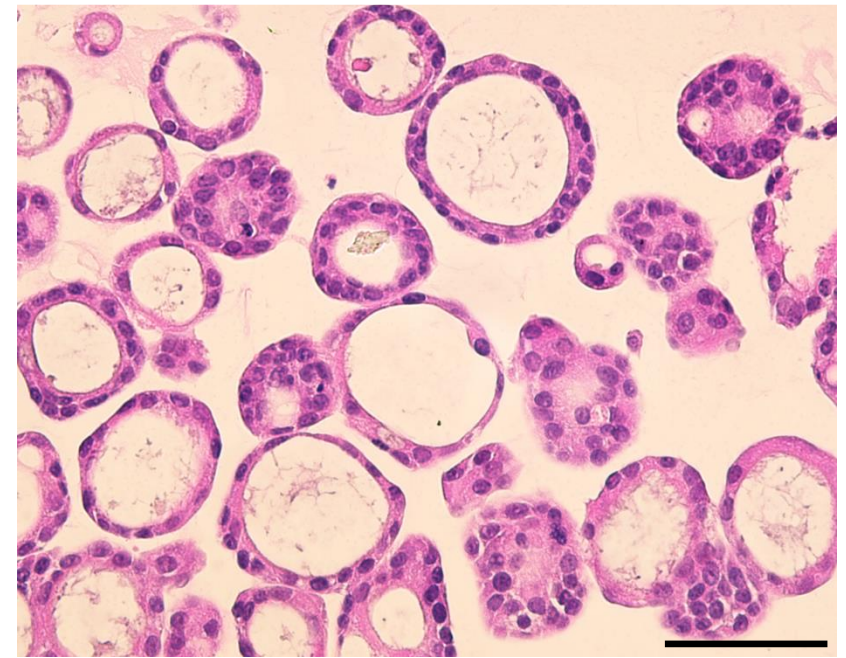
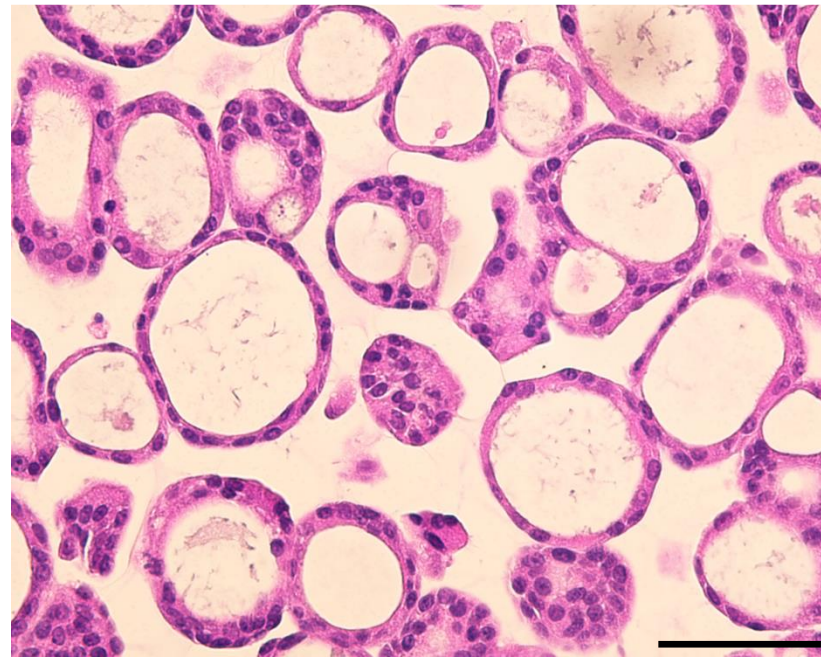
Scientific validation

- Pathology
- 3D Imaging → TomoCube
- Single cell RNA sequencing
- Next Generation Sequencing
- Mechanotransduction (Matrix stiffness)
- Culture media optimization, Co-culture

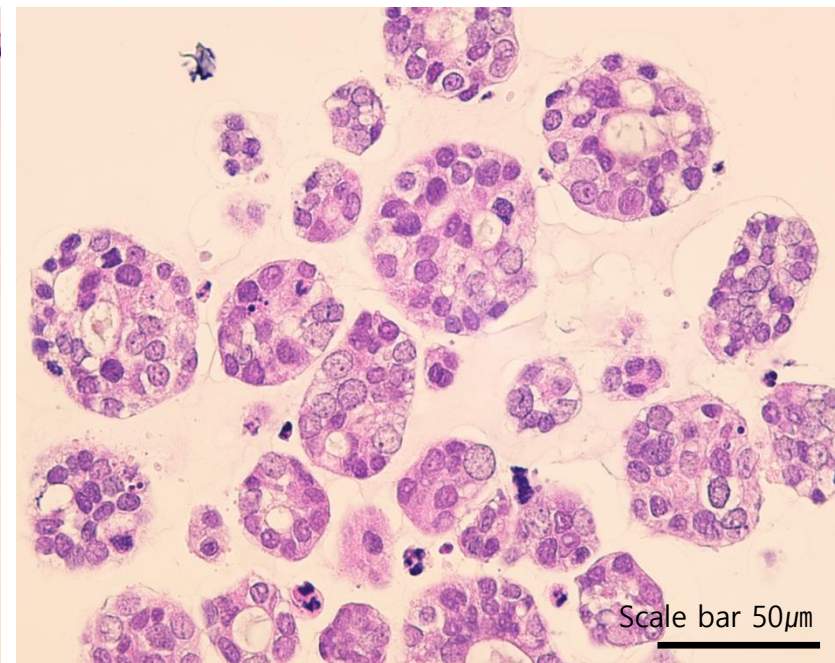
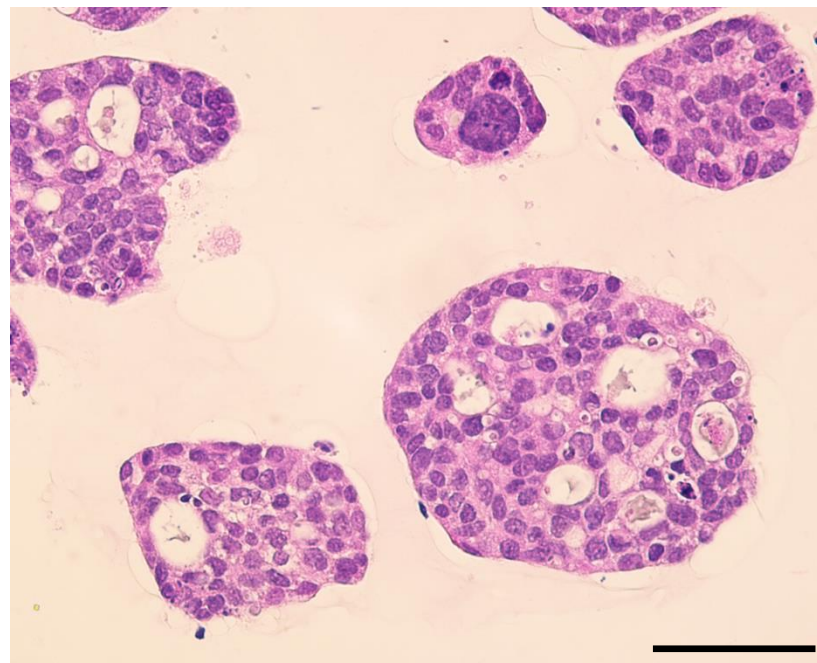
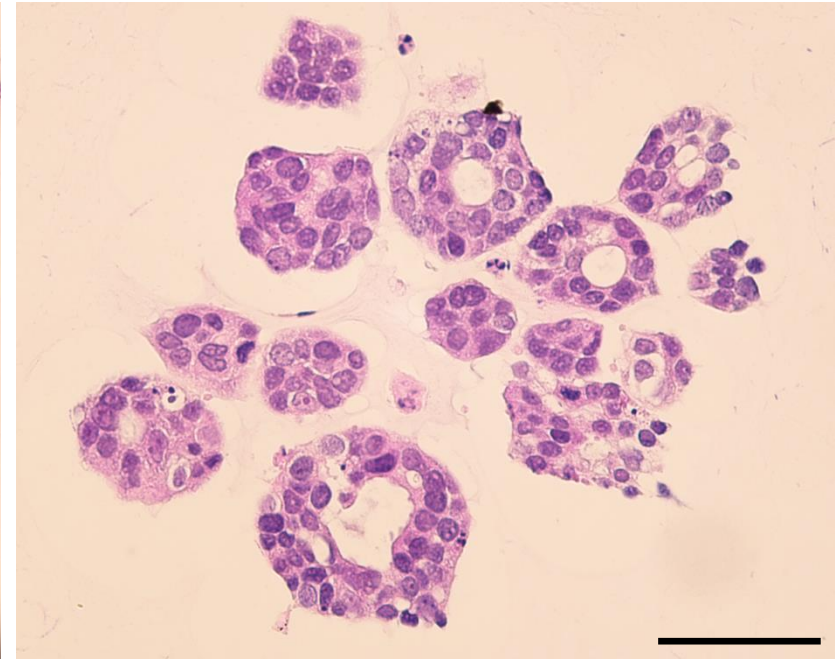
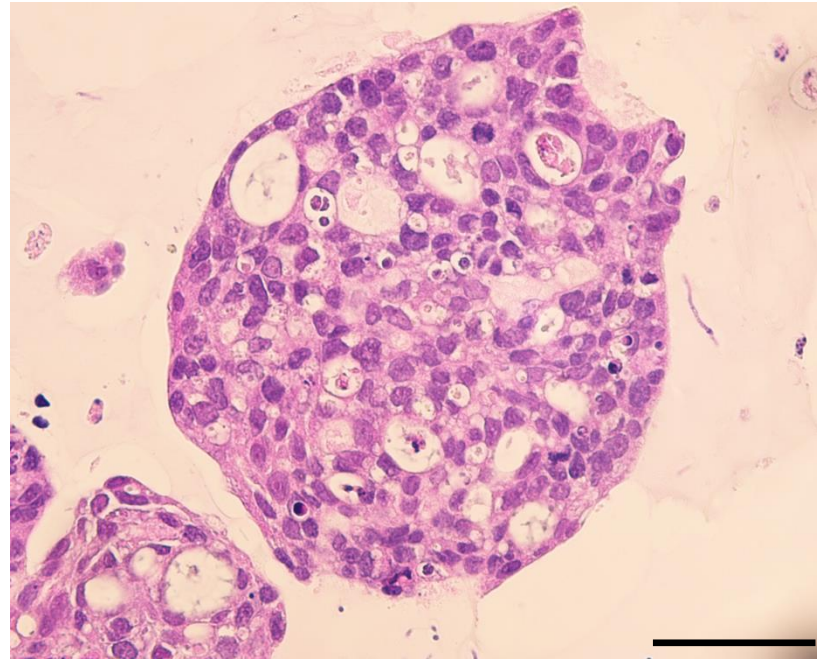
H & E staining of normal lung organoid and lung cancer organoid



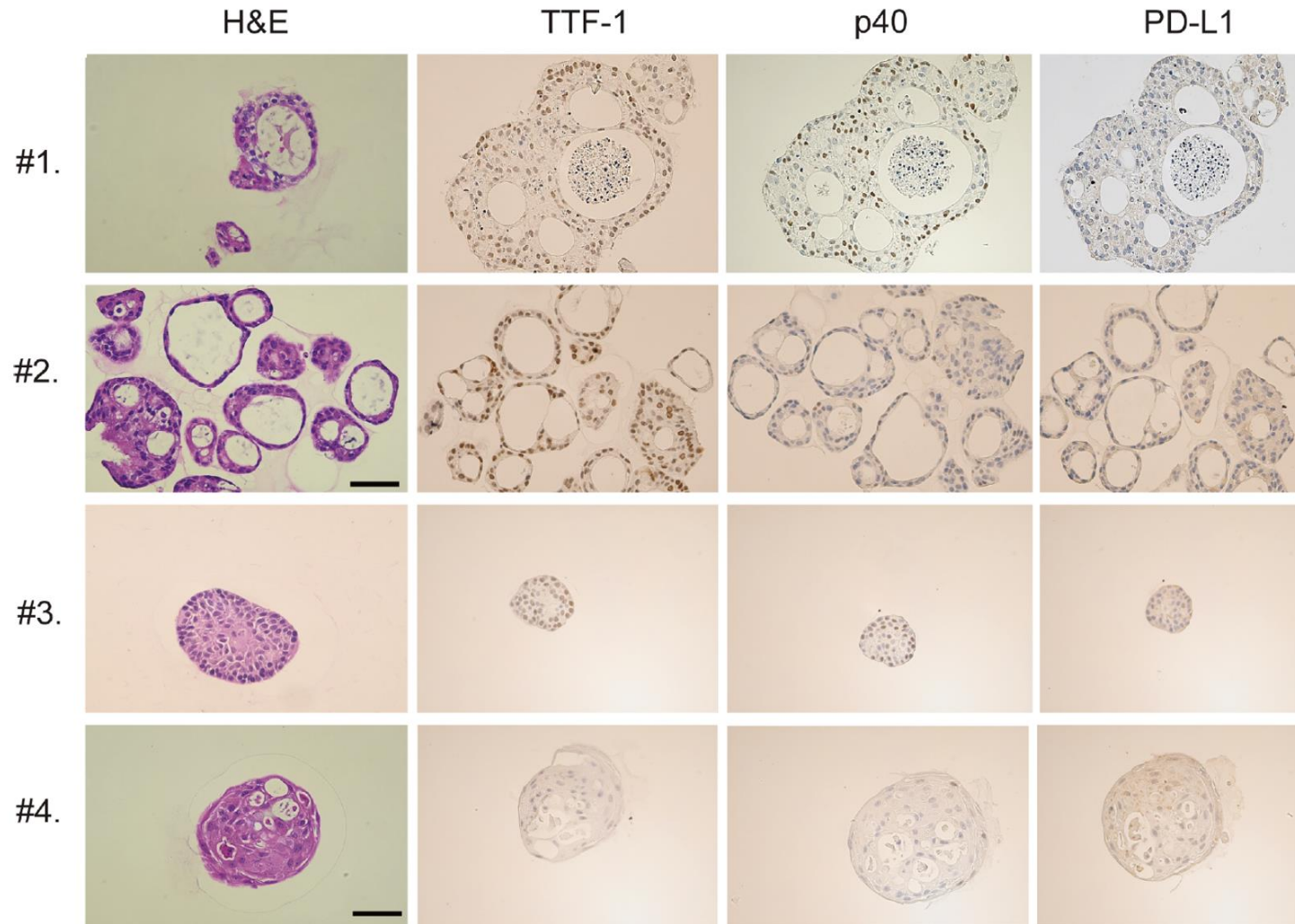
**Normal
lung organoid**



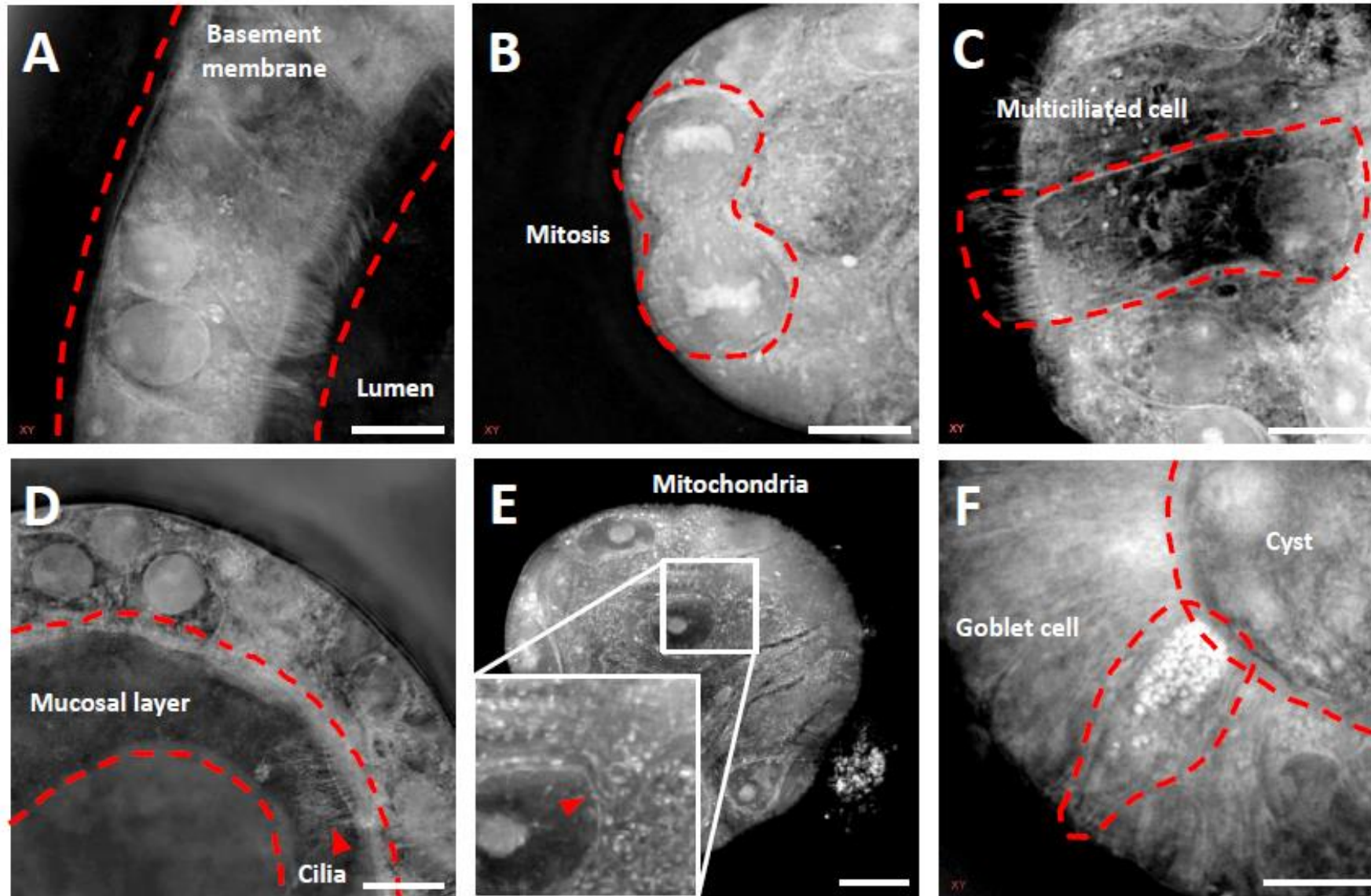
Lung cancer organoid



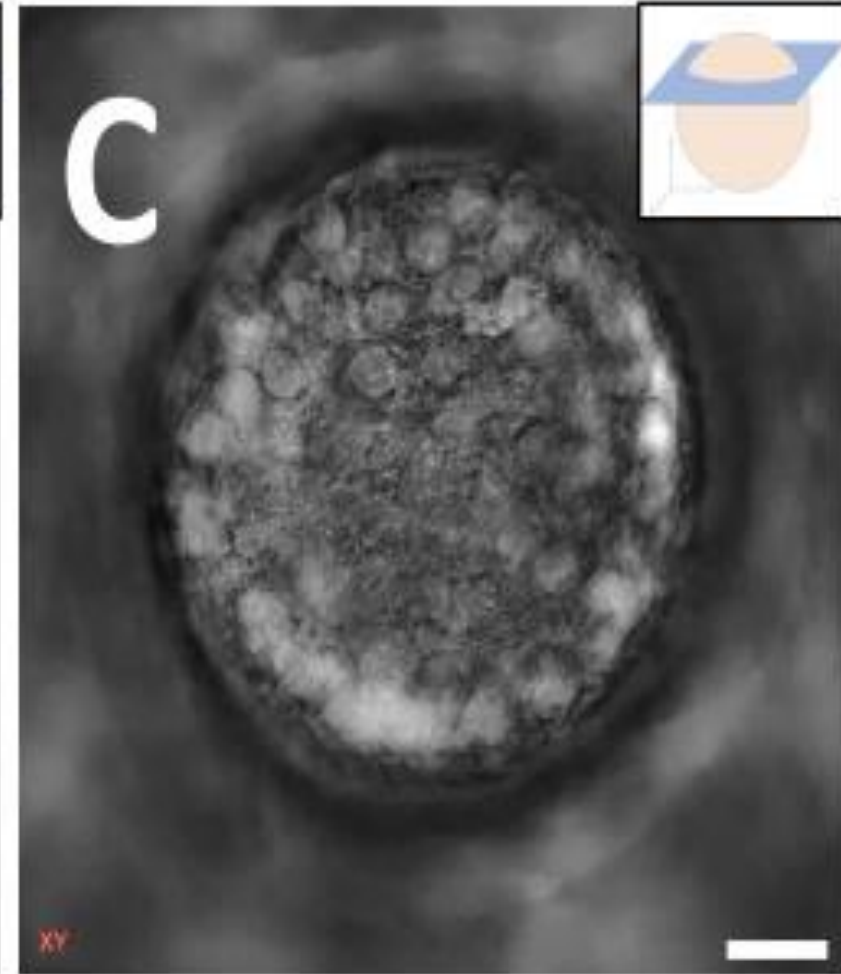
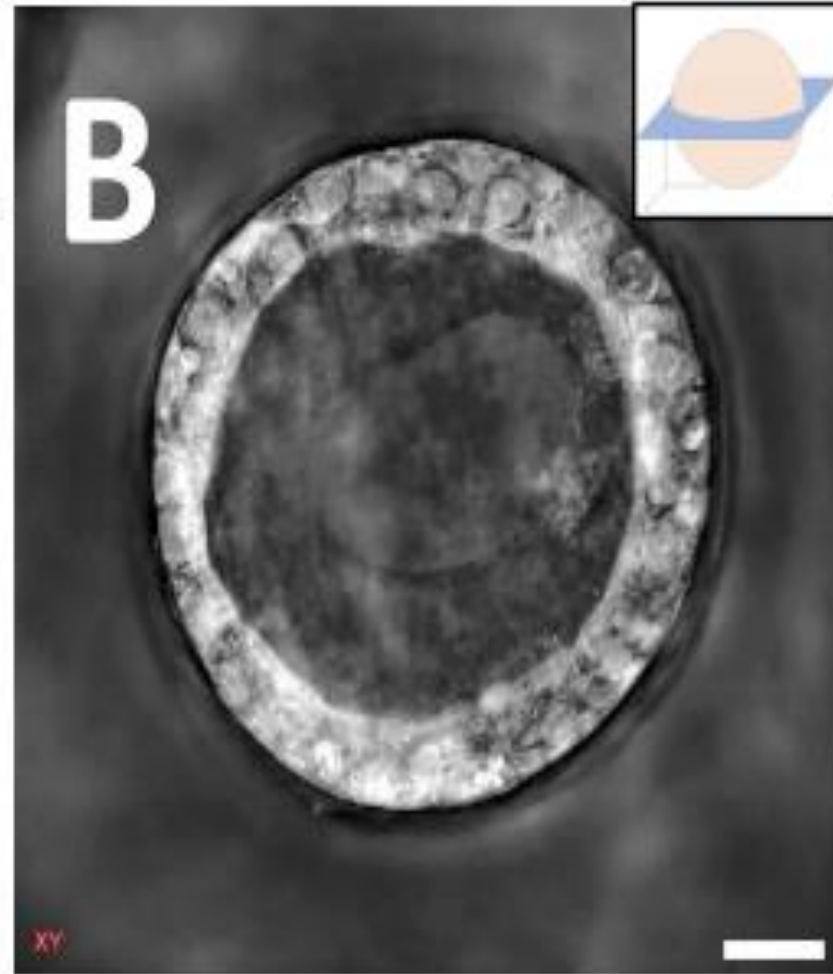
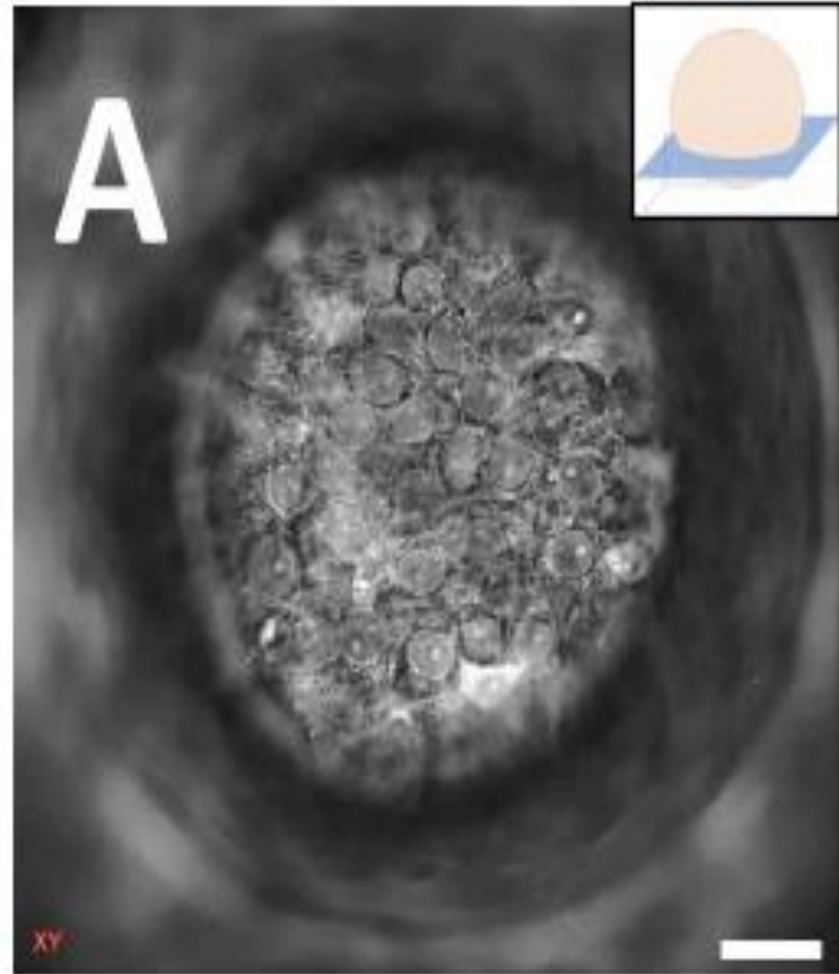
The representative pictures of H&E staining and IHC staining of lung cancer organoids



Diverse subcellular-level features of lung organoid

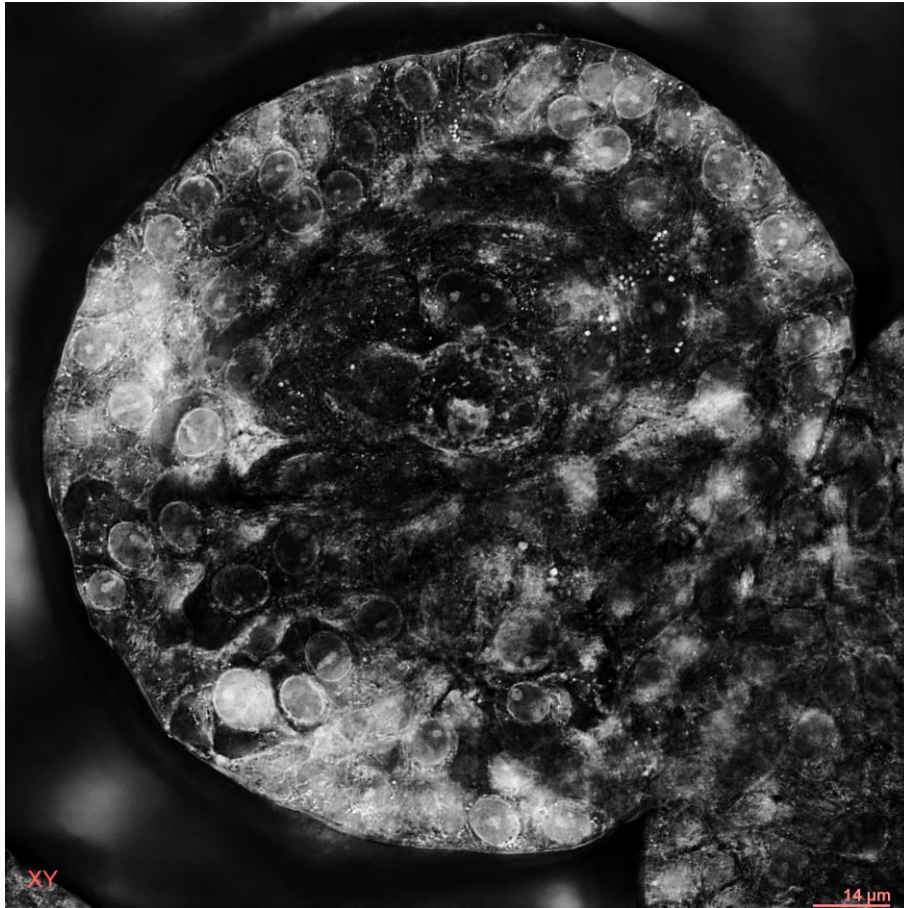


Fixed Sample (Mature lung organoid)

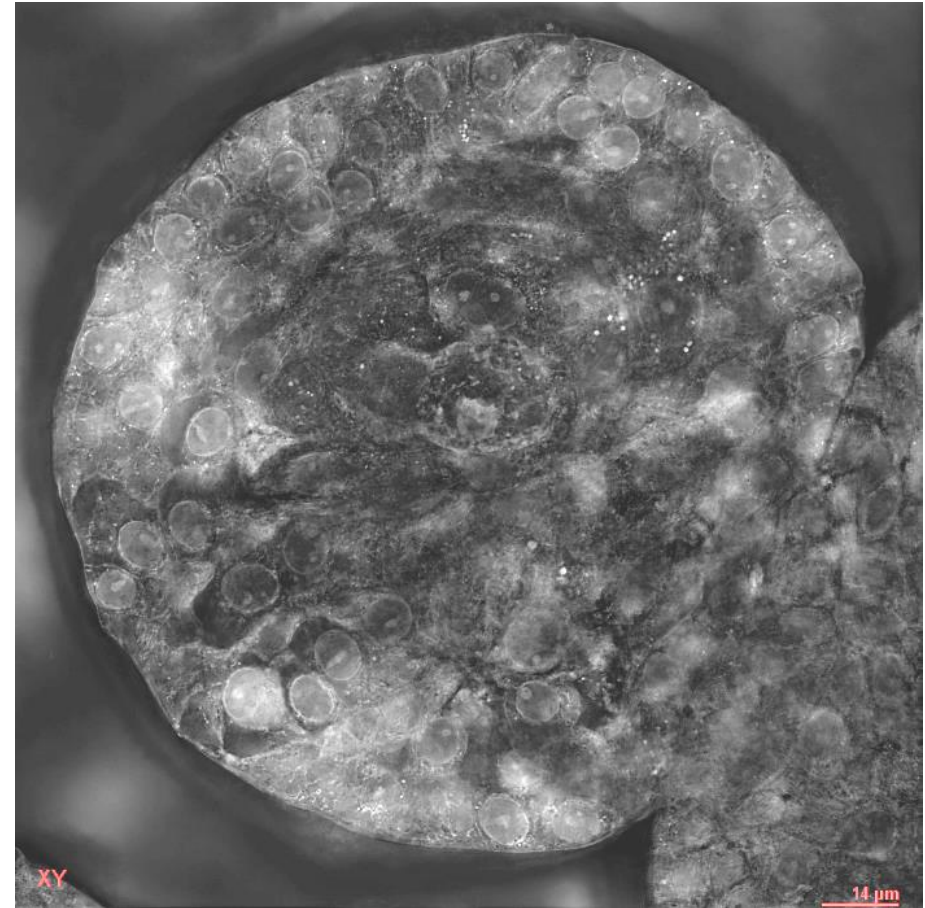


Tumor small biopsy lung organoid; Fixed

XY Slice

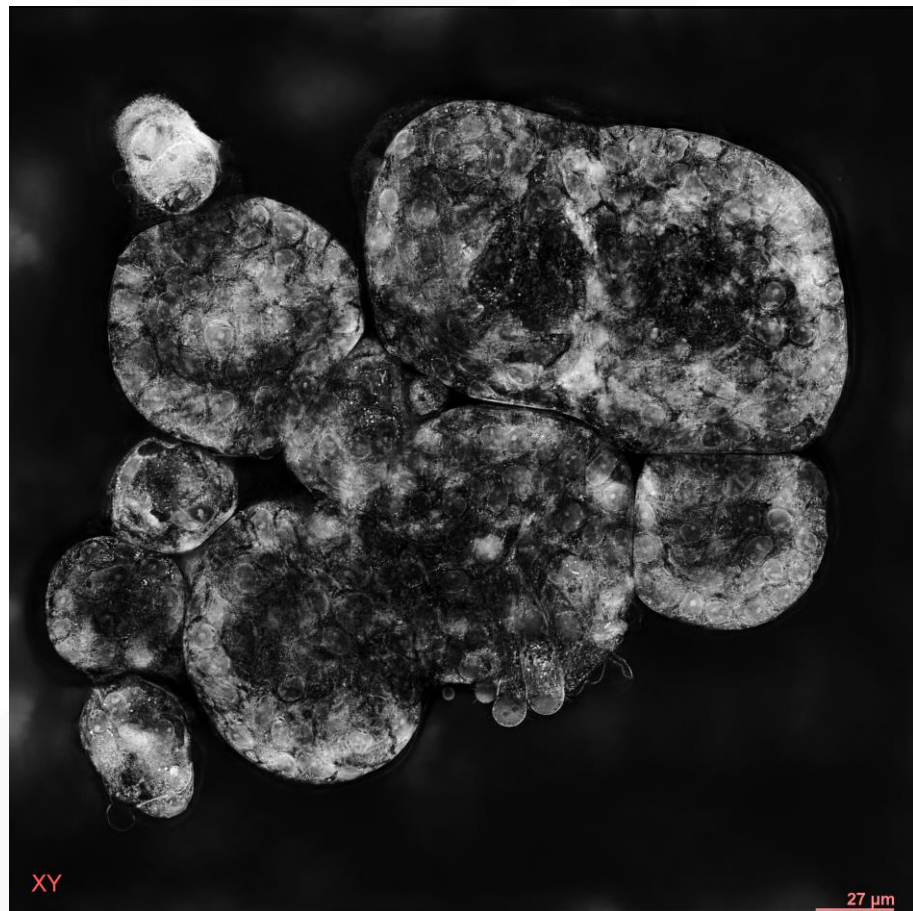


XY Movie

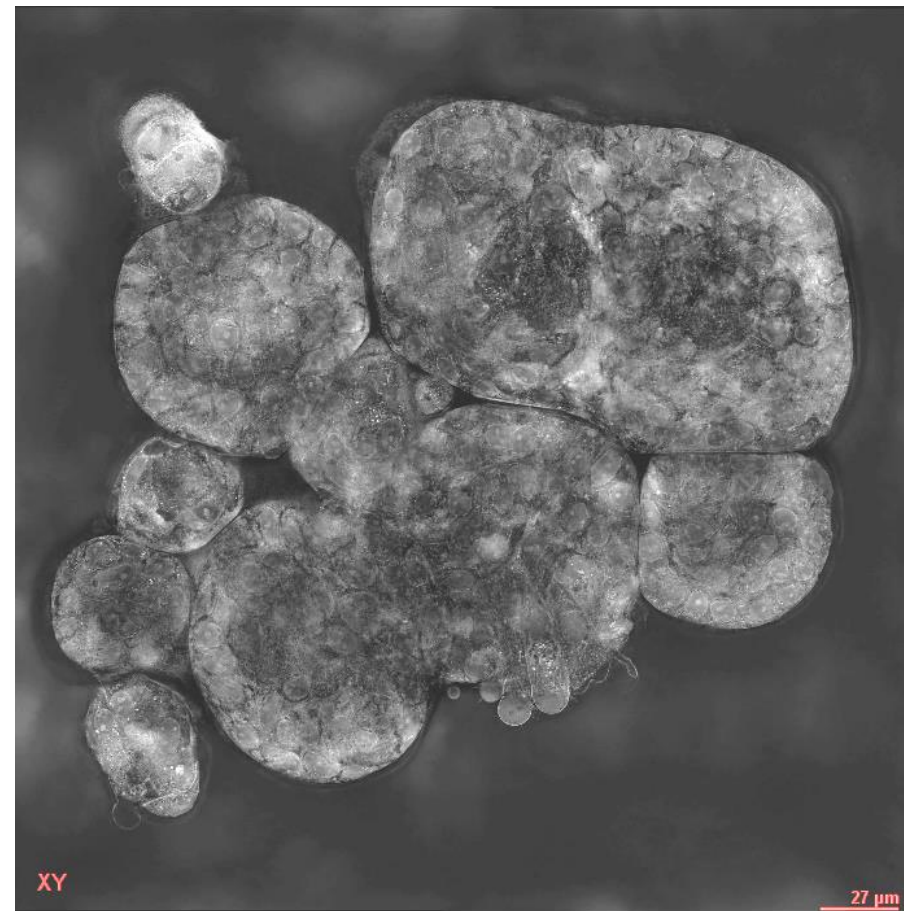


Tumor small biopsy lung organoid; Fixed

XY Slice

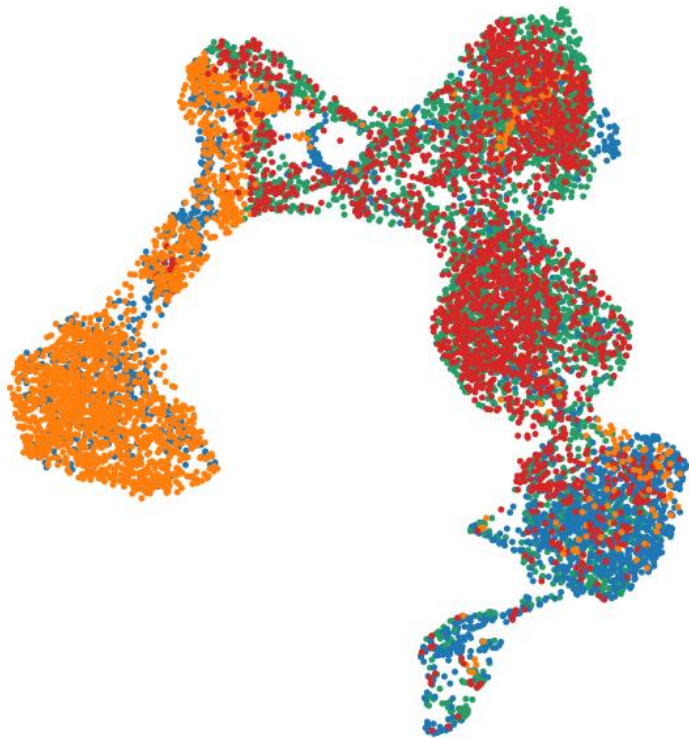


XY Movie



Single cell RNA sequencing of Lung cancer organoid

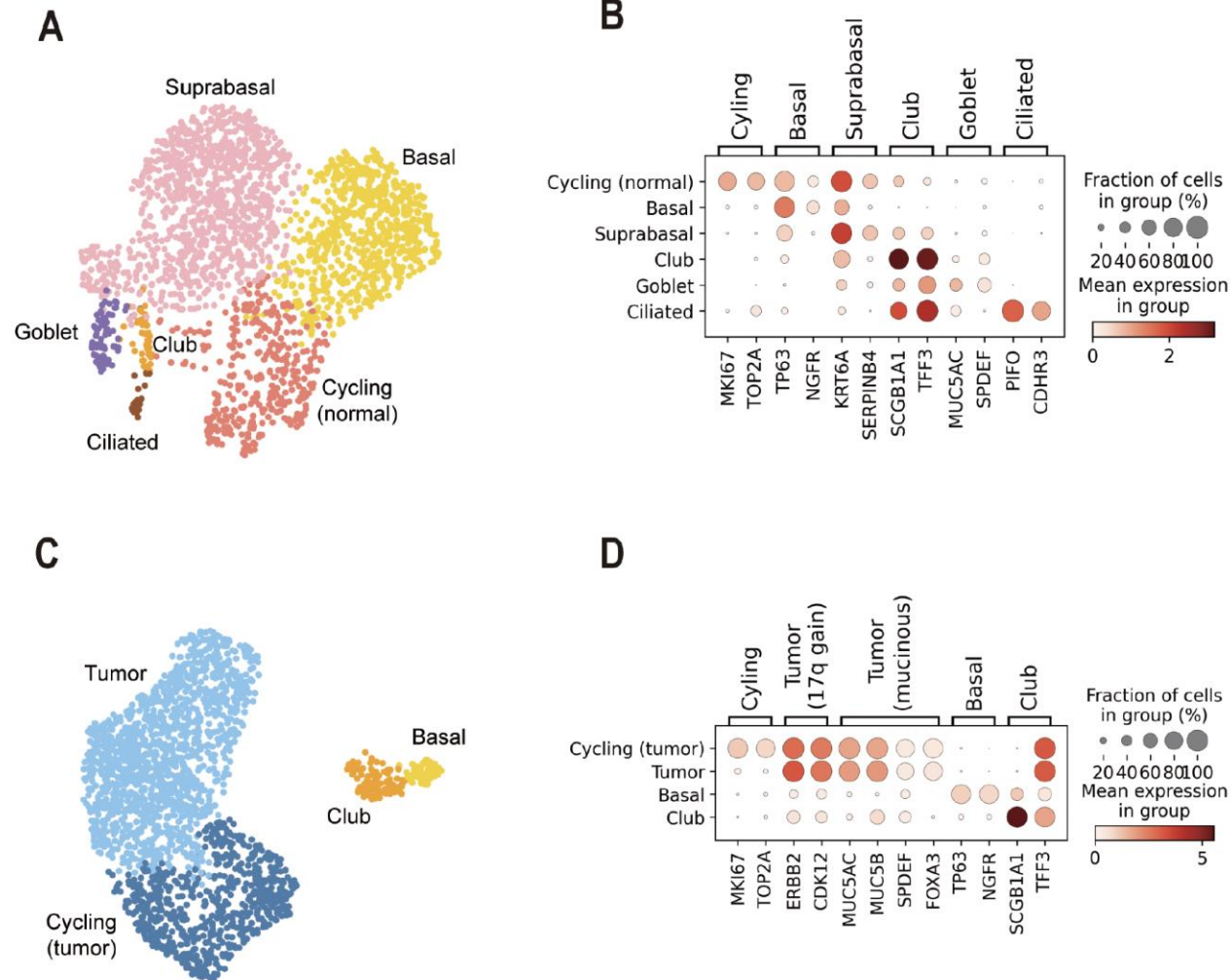
sample



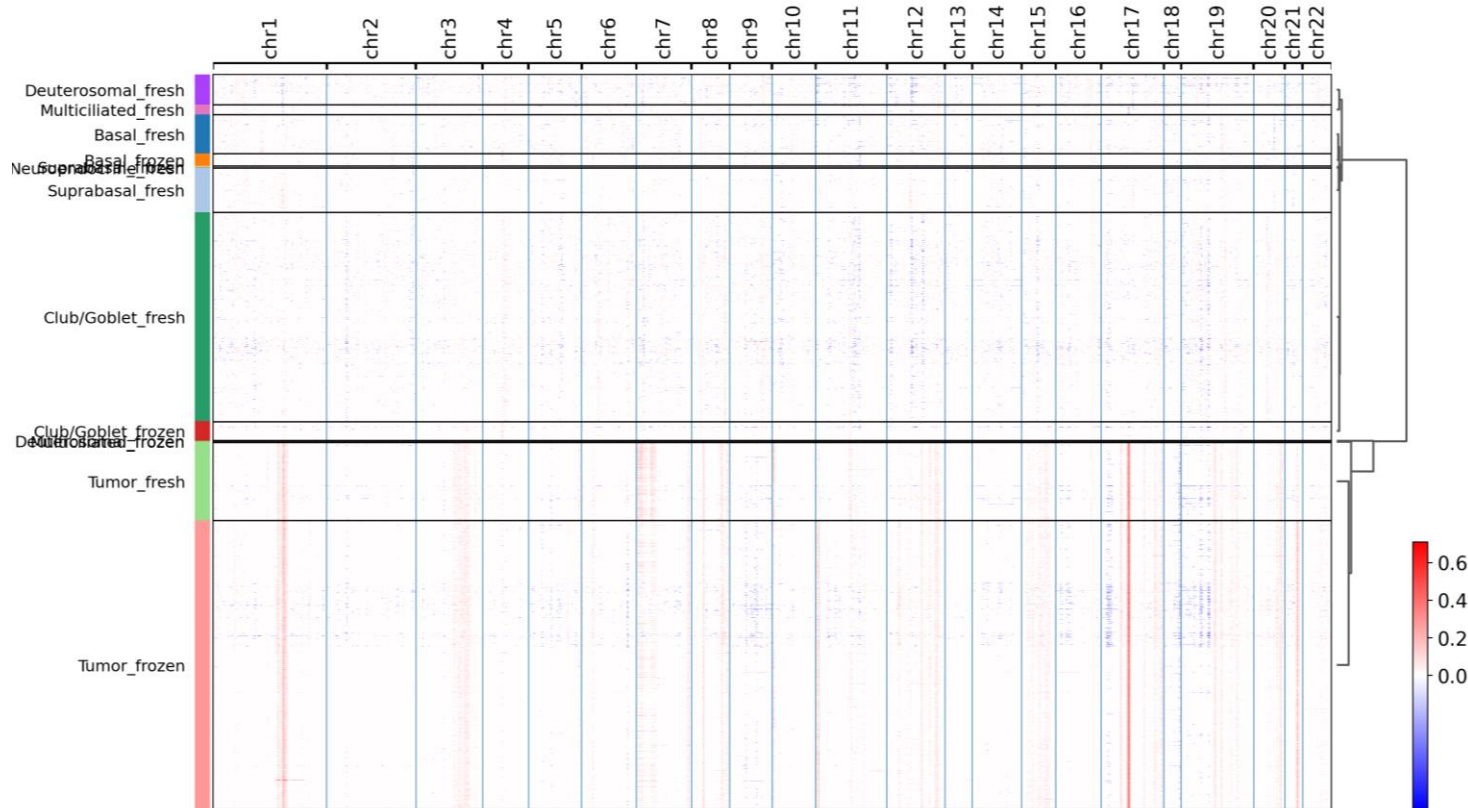
Total 9,580 cells after filtering

| | | |
|-------------------|---------------|----------------|
| ● SB_182-2_fresh | (2,221 cells) | Adenocarcinoma |
| ● SB_182-2_frozen | (2,080 cells) | |
| ● SB_195-2_fresh | (3,141 cells) | Non-neoplastic |
| ● SB_195-2_frozen | (2,138 cells) | |

Single cell RNA sequencing of Lung cancer organoid



Detection of rare mutation by using lung cancer organoid



SB#182-2 (Adenocarcinoma, RLL)



chr17 EBBR2 amplification

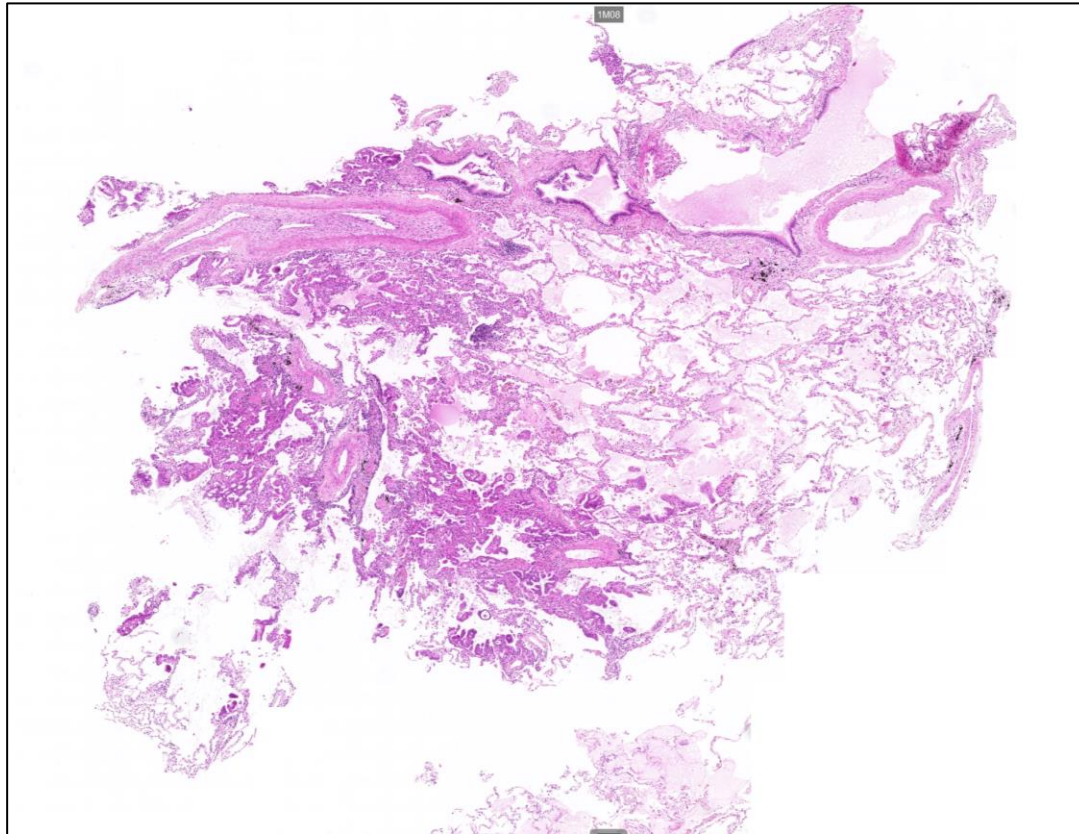
```

* S2022-5468 (block C); ADENOCARCINOMA, primary versus metastatic
* 비유전성 고형암 유전자 패널 검사 (TMB/MSI) (RNA 미포함) (의료법인 녹십자의
  료재단) (결과 이미지 추가됨)

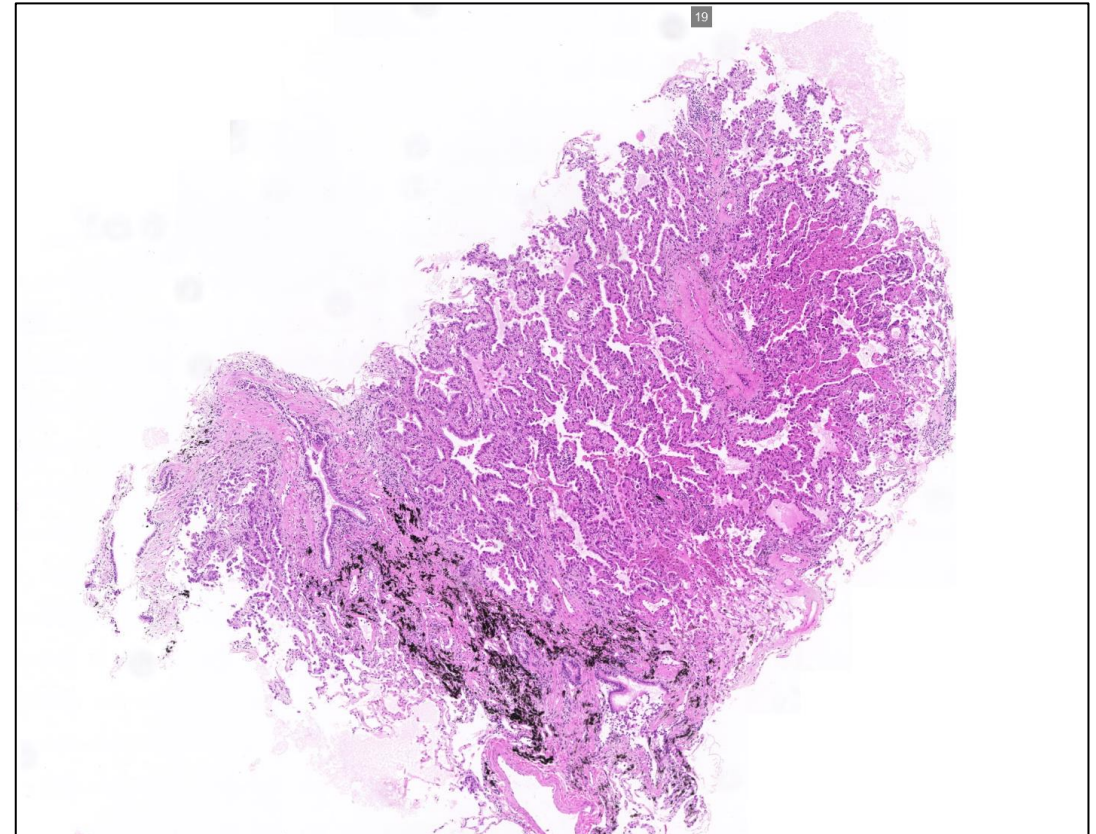
- Tier 1: Variants of strong clinical significance: No variant
- Tier 2: Variants of potential clinical significance: 2
  Gene      DNA      Protein
  1) ERBB2  Amplification  95.2 copy number gain*
  2) TP53   c.920-1G>A    p.?
- Tier 3: Variants of unknown clinical significance: No variant

- TMB score (백만 base pair 당 검출된 mutation의 개수): 5.67 Mutations/Mb
- MSI status: MSS
    
```

Purity of cancer cell in small biopsy tissue



Small tumor with many normal cells
→ LCO with low cancer cell proportion



Large tumor, advanced lung cancer
→ LCO with nearly 100% cancer cells

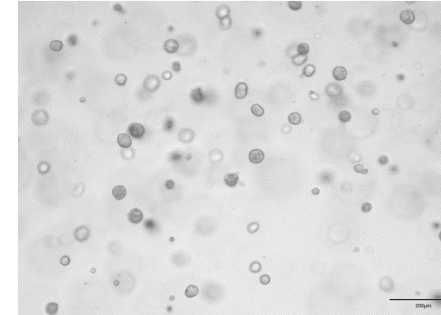
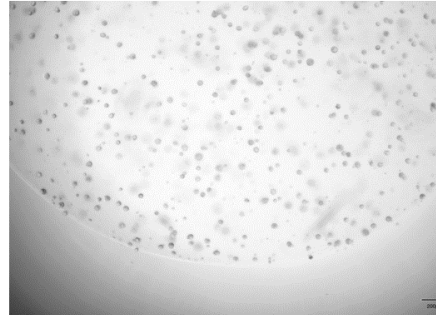
Co-culture with fibroblast

*96well plate – 7일차

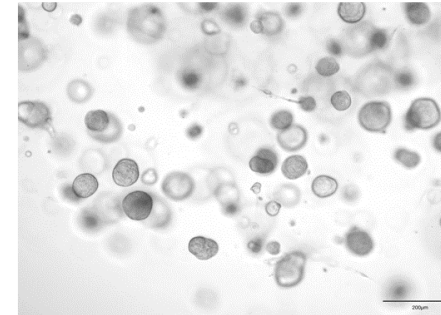
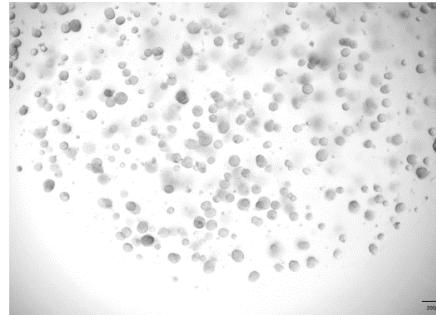
저배율

고배율

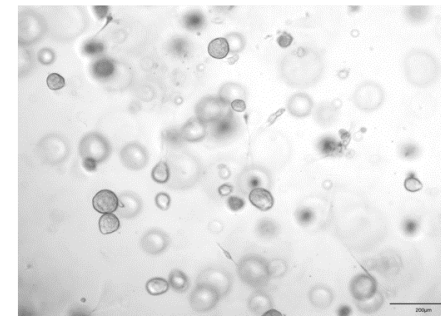
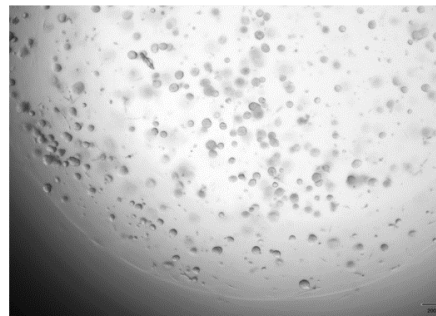
Normal lung organoid
 2×10^3 cell



Normal lung organoid
 2×10^3 cell
 + Normal fibroblast
 2×10^3 cell



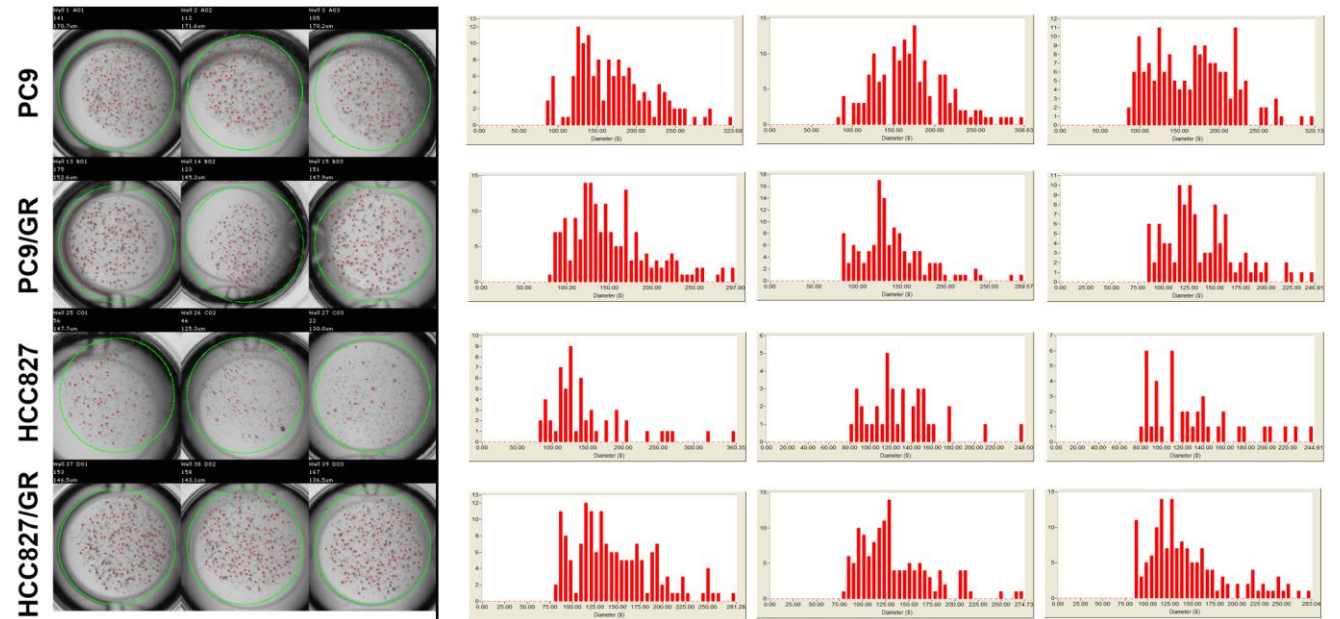
Normal lung organoid
 2×10^3 cell
 + Normal fibroblast
 4×10^3 cell



폐 오가노이드 크기 및 개수 변화 확인



GelCount™
Mammalian-cell colony, spheroid and organoid counter



Drug screening (Micropillar)

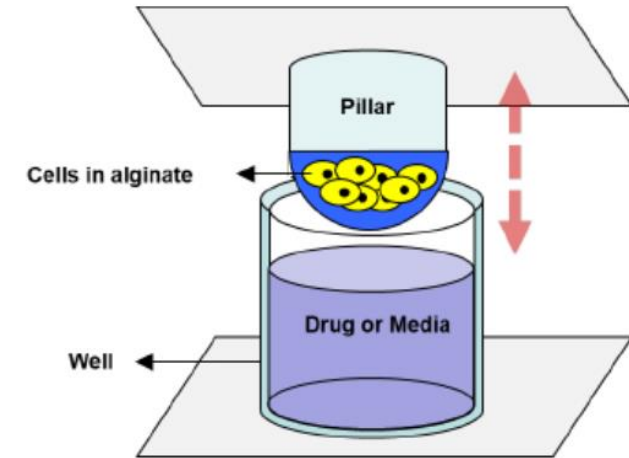
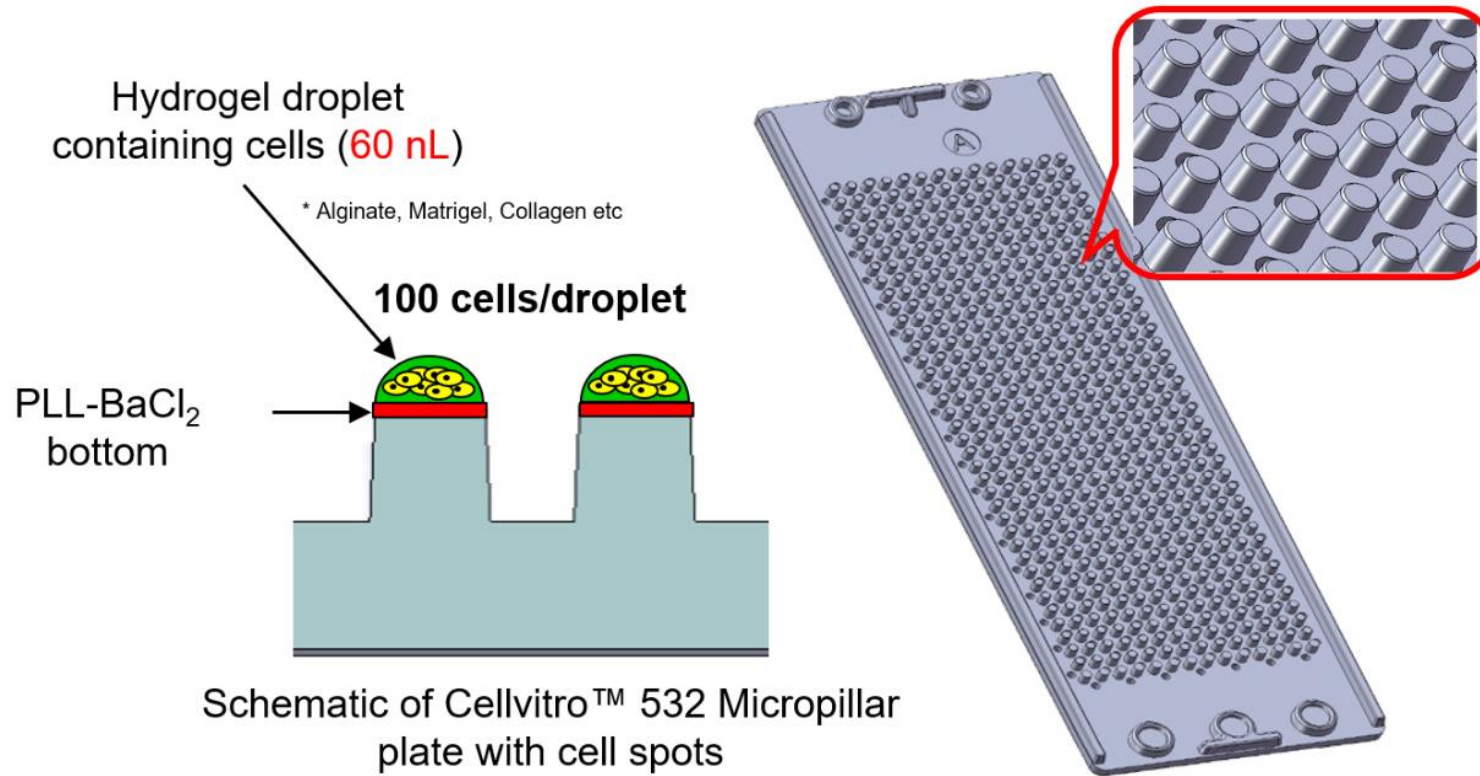
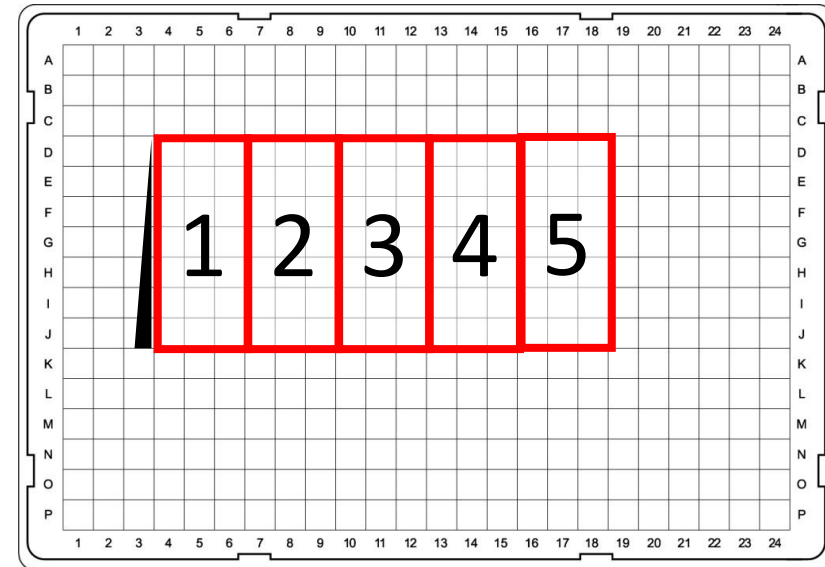
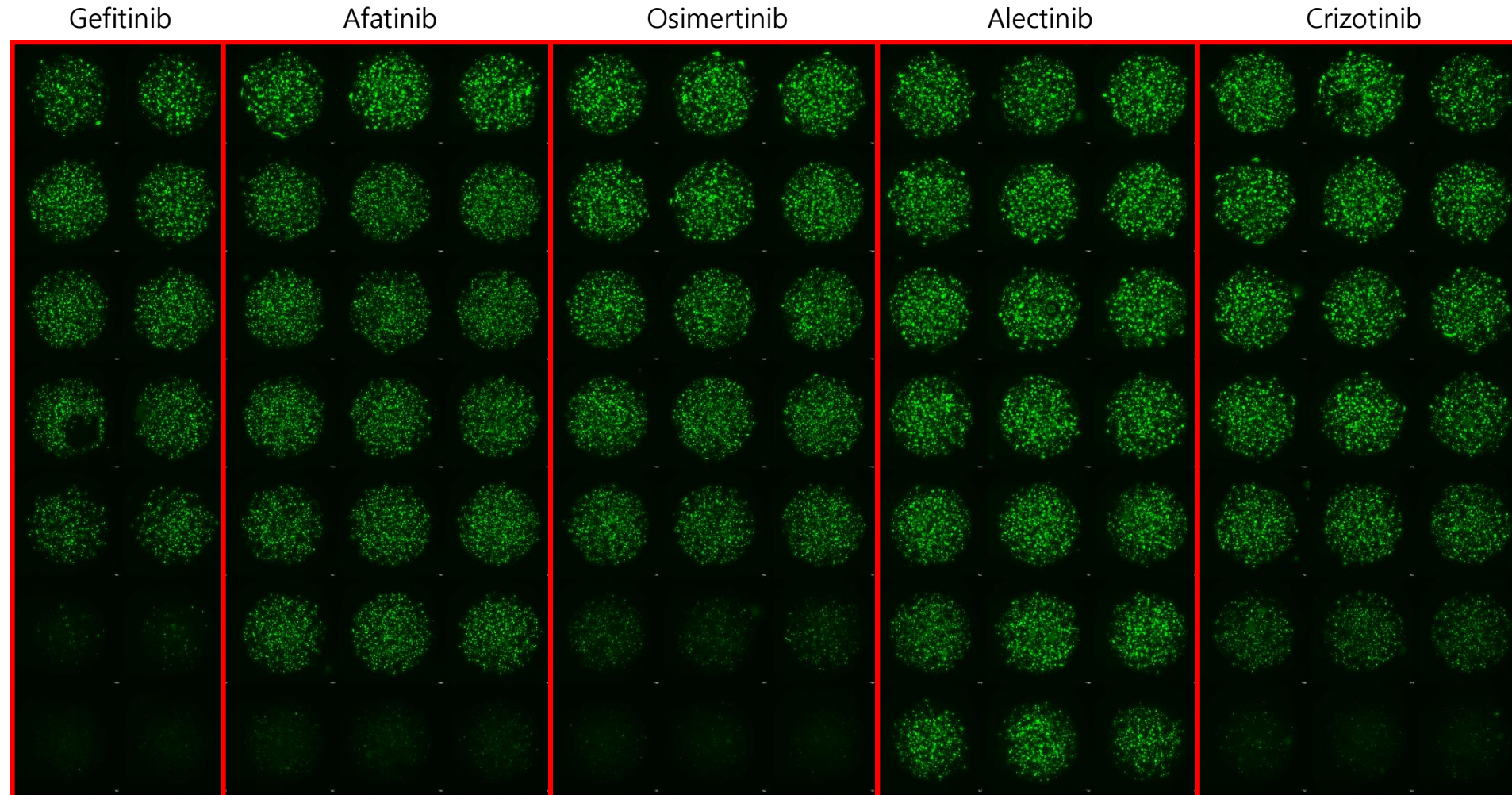


Plate layout

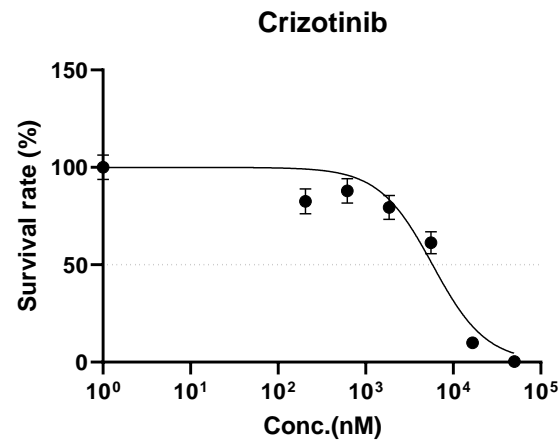
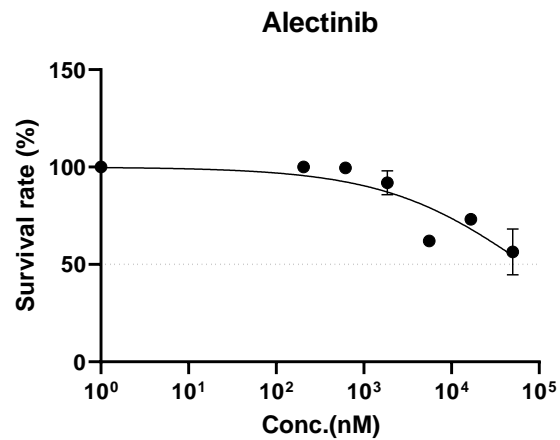
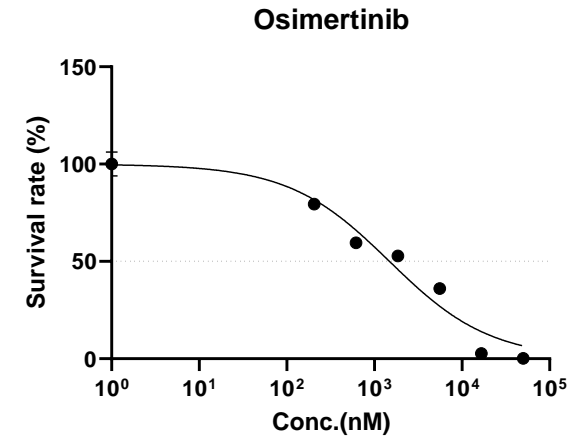
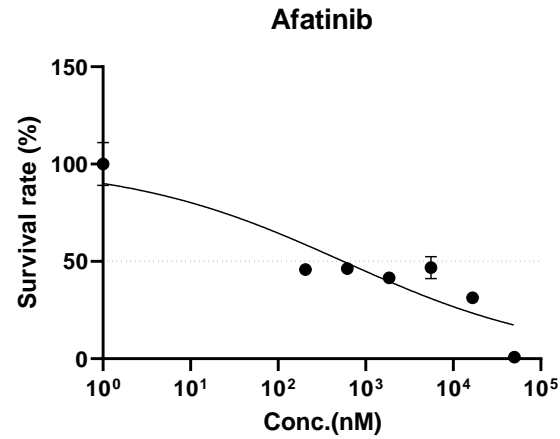
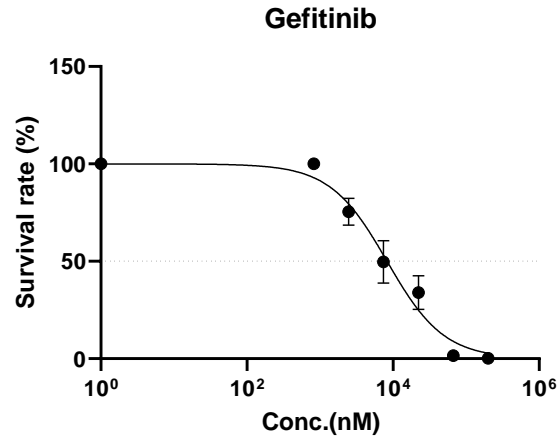
| 약물 번호 | 약물 | 최고농도 | Solvent | Dilution 비 |
|-------|-------------|-------|---------|------------|
| 1 단독1 | Gefitinib | 200uM | DMSO | 3 |
| 2 단독2 | Afatinib | 50 uM | DMSO | 3 |
| 3 단독3 | Osimertinib | 50 uM | DMSO | 3 |
| 4 단독4 | Alectinib | 50 uM | DMSO | 3 |
| 5 단독5 | Crizotinib | 50 uM | DMSO | 3 |



Results



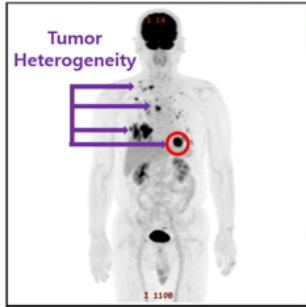
Results



| | IC50(uM) | AUC |
|-------------|----------|-------|
| Gefitinib | 8.3 | 148.3 |
| Afatinib | 0.79 | 125.1 |
| Osimertinib | 1.8 | 133.9 |
| Alectinib | >50 | 240.9 |
| Crizotinib | 7.1 | 177.2 |

오가노이드 클러스터

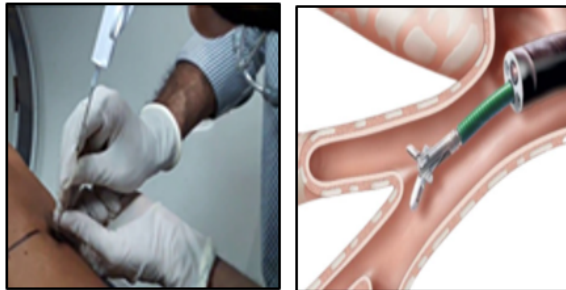
비수술 조직 유래 폐암 오가노이드 클러스터의 임상적 활용 플랫폼 구축



- Multi-site, Multi-time small biopsy (High purity of cancer cell)
- Enzyme free CNUH Digestion
- Optimized organoid culture media (containing Noggin, FGF-7, FGF-10)



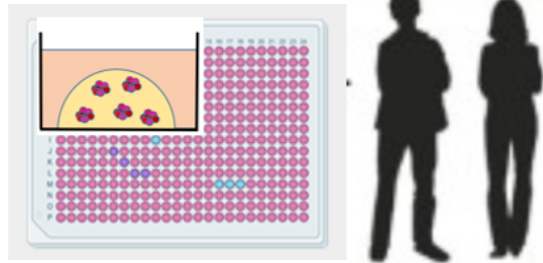
전 병기 폐암 오가노이드
뱅크 및 클러스터 구축



폐암 오가노이드의
공간적, 시간적 이질성 분석

폐암 오가노이드와 면역, 혈관,
간엽세포 공동 배양 시스템 구축

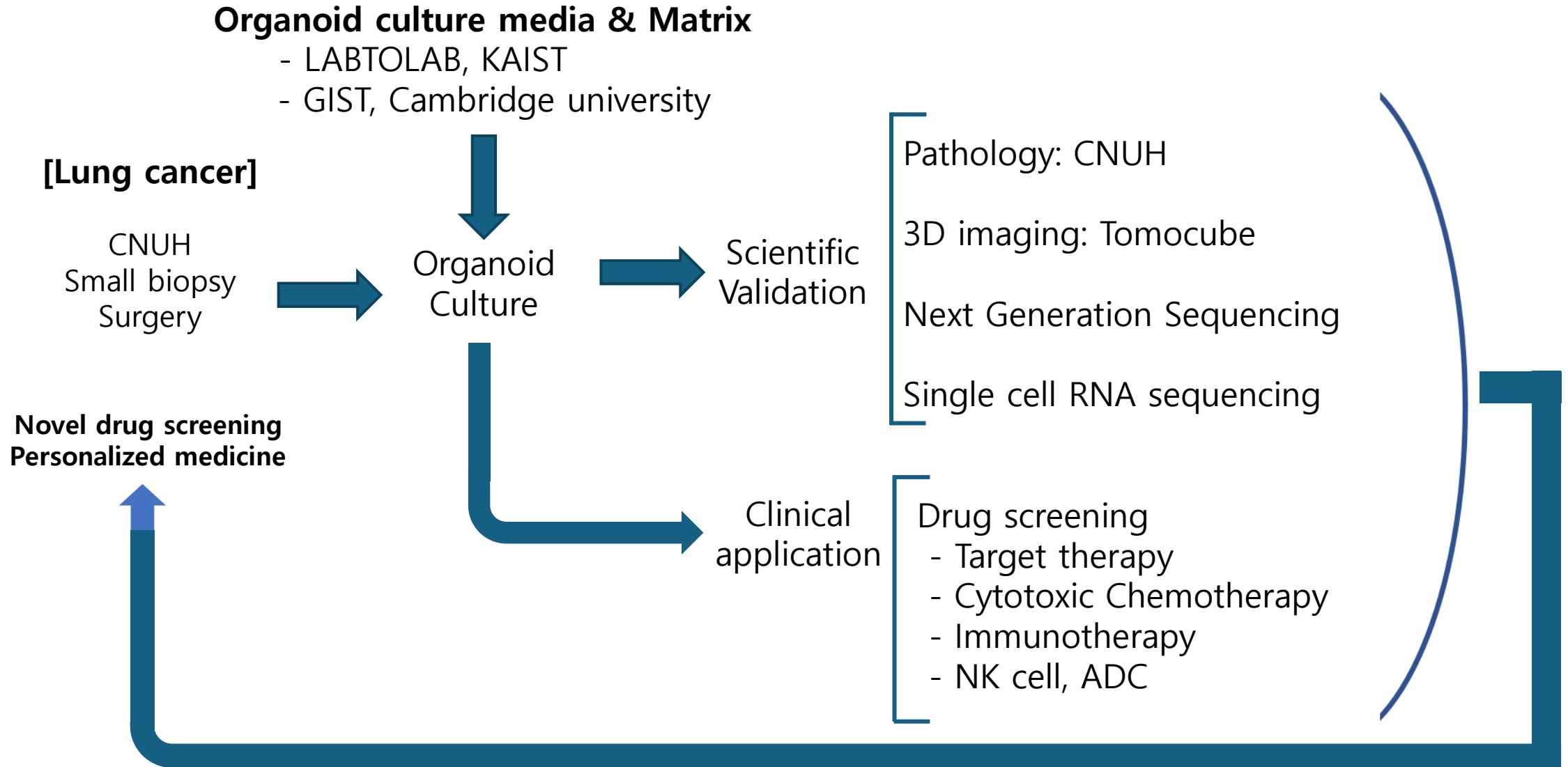
폐암 오가노이드의
배양 매트릭스 최적화



임상 진료에 활용가능한
폐암 오가노이드 활용 플랫폼

폐암의 항암제 내성 극복
고도화된 개인 맞춤형 치료

폐암 오가노이드 임상 활용 플랫폼 구축



Review

New Insights into the Clinical Implications of Yes-Associated Protein in Lung Cancer: Roles in Drug Resistance, Tumor Immunity, Autophagy, and Organoid Development

 Geon Yoo ^{1,†}, Dongil Park ^{2,†}, Yoonjoo Kim ^{2,†} and Chaeuk Chung ^{1,2,3,*}

- Clinical Research Division, National Institute of Food and Drug Safety Evaluation, Cheongju 28159, Chungcheongbuk-do, Korea; geonyoo@korea.kr
 - Division of Pulmonology and Critical Care Medicine, Department of Internal Medicine, College of Medicine, Chungnam National University, Daejeon 35015, Korea; rahms@cnuh.co.kr (D.P.); k1206@medu.chung.ac.kr (Y.K.)
 - Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon 35015, Korea
- * Correspondence: universe7903@gmail.com
 † These authors contributed equally to this paper.

Simple Summary: Innovative advancements in lung cancer treatment have developed over the past decade with the advent of targeted and immune therapies. Yes-associated protein (YAP), an effector of the Hippo pathway, promotes the resistance of these targeted drugs and modulates tumor immunity in lung cancer. YAP is involved in autophagy in lung cancer and plays a prominent role in forming the tubular structure in lung organoids and alveolar differentiation. In this review, we discuss the central roles of YAP in lung cancer and present YAP as a novel target for treating resistance to targeted therapies and immunotherapies in lung cancer.

Abstract: Despite significant innovations in lung cancer treatment, such as targeted therapy and immunotherapy, lung cancer is still the principal cause of cancer-associated death. Novel strategies to overcome drug resistance and inhibit metastasis in cancer are urgently needed. The Hippo pathway and its effector, Yes-associated protein (YAP), play crucial roles in lung development and alveolar differentiation. YAP is known to mediate mechanotransduction, an important process in lung homeostasis and fibrosis. In lung cancer, YAP promotes metastasis and confers resistance against chemotherapeutic drugs and targeted agents. Recent studies revealed that YAP directly controls the expression of programmed death-ligand 1 (PD-L1) and modulates the tumor microenvironment (TME). YAP not only has a profound relationship with autophagy in lung cancer but also controls alveolar differentiation, and is responsible for tubular structure formation in lung organoids. In this review, we discuss the various roles and clinical implications of YAP in lung cancer and propose that targeting YAP can be a promising strategy for treating lung cancer.

Keywords: YAP; TAZ; Hippo pathway; lung cancer; drug-resistance; EGFR-TKI; PD-L1; autophagy; organoid

1. Introduction

The Hippo signaling pathway has been one of the most actively studied pathways in biomedicine over the past decade [1–3]. It was first discovered in *Drosophila*, where it regulates cell differentiation and proliferation during development [4,5]. Studies in mice later revealed that it is highly involved in tumorigenesis and metastasis [2]. Yes-associated protein (YAP) is a downstream transcriptional co-activator of the Hippo pathway. It controls cell proliferation and apoptosis by regulating the transcription of genes that control DNA replication, DNA repair, apoptosis, and metabolism [6]. YAP also cooperates with other transcription factors, including AP1, β -catenin, and cytokines such as TGF- β , to control many cellular functions [3,7]. Furthermore, it senses and responds to mechanical

Review

Scientific Validation and Clinical Application of Lung Cancer Organoids

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- Division of Pulmonology and Critical Care Medicine, Department of Internal Medicine, College of Medicine, Chungnam National University, Daejeon 34134, Korea; zicz02@naver.com (D.L.); misspms@naver.com (Y.K.)
 - Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon 35015, Korea
- * Correspondence: universe7903@gmail.com
 † These authors contribute equally to this work.

Abstract: Lung cancer organoid (LCO) is a novel model of lung cancer that facilitates drug screening. However, the success rate of LCOs varies from 7% to 87%, and the culture medium compositions are markedly different. Airway organoid media can be used for LCO cultures, but this promotes the overgrowth of normal cell organoids especially in LCOs from intrapulmonary lesions. Several modified media are specifically utilized for promoting the cancer cell's growth. For culturing high-purity LCOs, cancer cells from metastatic lesions and malignant effusions are used. Recently, single-cell RNA sequencing has identified previously unknown cell populations in the lungs and lung cancer. This sequencing technology can be used to validate whether the LCO recapitulates the heterogeneity and functional hierarchy of the primary tumor. Several groups have attempted to culture LCOs with mesenchymal cells and immune cells to recapitulate the tumor microenvironment. Disease modeling using LCO provides novel insight into the pathophysiology of lung cancer and enables high-throughput screening for drug discovery and prognosis prediction. An LCO model would help to identify new concepts as a basis for lung cancer targeting by discovering innovative therapeutic targets.

Keywords: lung neoplasms; organoids; culture media; coculture techniques; clinical study

1. Introduction

Despite innovations in targeted therapy and immunotherapy in the last decade, lung cancer remains the most common cause of cancer related death [1–3]. Tumor heterogeneity and drug resistance hamper the treatment of lung cancer [4]. To identify novel strategies to overcome these problems, faithful lung cancer models are needed for preclinical research.

Organoids are three-dimensional cellular complexes that originate from embryonic stem cells, induced pluripotent stem (iPS) cells, or adult stem/progenitor cells [5–8]. They mimic the basic structure and function of the primary organ. Organoids are composed of several cell populations whereas spheroids are simple collections of one cell type [6,7]. Organoid culture has opened new avenues for both basic and translational medicine. Using an organoid model, disease pathophysiology can be investigated, and novel treatment strategies can be evaluated.

Cancer organoids show promise for cancer research by recapitulating the tumor characteristics and heterogeneity [9]. The use of lung cancer organoids (LCOs) enable reliable high-throughput drug screening [10–12]. However, the success rate of LCO culture, the culture medium composition, and tissue processing differ among studies. Because the overgrowth of normal lung organoids is common in LCOs originating from surgical specimens, tumor cell purity is a critical issue for studies using LCOs. There is still controversy as to whether LCOs reflect tumor heterogeneity. Therefore, research aiming to improve LCO purity and validate the recapitulation of primary tumors is underway.



Citation: Lee, D.; Kim, Y.; Chung, C. Scientific Validation and Clinical Application of Lung Cancer Organoids. *Cells* **2021**, *10*, 3012. <https://doi.org/10.3390/cells10113012>

Academic Editor: Alessandra Mangia
 Received: 12 October 2021
 Accepted: 1 November 2021
 Published: 4 November 2021

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Cells **2021**, *10*, 3012. <https://doi.org/10.3390/cells10113012>

<https://www.mdpi.com/journal/cells>

Article

Cryobiopsy: A Breakthrough Strategy for Clinical Utilization of Lung Cancer Organoids

 Dongil Park ^{1,†}, Dahye Lee ^{1,†}, Yoonjoo Kim ¹, Yeonhee Park ², Yeon-Jae Lee ¹, Jeong Eun Lee ¹, Min-Kyung Yeo ³, Min-Woong Kang ⁴, Yooyoung Chong ⁴, Sung Joon Han ⁴, Jinwook Choi ⁵, Jong-Eun Park ⁶, Yongjun Koh ⁶, Jaehyeok Lee ⁷, YongKeun Park ^{7,8}, Ryul Kim ⁹, Jeong Seok Lee ^{6,9}, Jimin Choi ¹⁰, Sang-Hyun Lee ¹⁰, Bosung Ku ¹⁰, Da Hyun Kang ¹⁰ and Chaeuk Chung ^{1,*}

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Citation: Park, D.; Lee, D.; Kim, Y.; Park, Y.; Lee, Y.-J.; Lee, J.E.; Yeo, M.-K.; Kang, M.-W.; Chong, Y.; Han, S.J.; et al. Cryobiopsy: A Breakthrough Strategy for Clinical Utilization of Lung Cancer Organoids. *Cells* **2021**, *12*, 1854. <https://doi.org/10.3390/cells12141854>

Academic Editor: Liwu Fu

Received: 9 May 2023
 Revised: 5 July 2023
 Accepted: 7 July 2023
 Published: 14 July 2023



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Abstract: One major challenge associated with lung cancer organoids (LCOs) is their predominant derivation from surgical specimens of patients with early-stage lung cancer. However, patients with advanced lung cancer, who are in need of chemotherapy, often cannot undergo surgery. Therefore, there is an urgent need to successfully generate LCOs from biopsy specimens. Conventional lung biopsy techniques, such as transthoracic needle biopsy and forceps biopsy, only yield small amounts of lung tissue, resulting in a low success rate for culturing LCOs from biopsy samples. Furthermore, potential complications, like bleeding and pneumothorax, make it difficult to obtain sufficient tissue. Another critical issue is the overgrowth of normal lung cells in later passages of LCO culture, and the optimal culture conditions for LCOs are yet to be determined. To address these limitations, we attempted to create LCOs from cryobiopsy specimens obtained from patients with lung cancer ($n = 113$). Overall, the initial success rate of establishing LCOs from cryobiopsy samples was 40.7% ($n = 46$). Transbronchial cryobiopsy enables the retrieval of significantly larger amounts of lung tissue than bronchoscopic forceps biopsy. Additionally, cryobiopsy can be employed for peripheral lesions, and it is aided via radial endobronchial ultrasonography. This study significantly improved the success rate of LCO culture and demonstrated that the LCOs retained characteristics that resembled the primary tumors. Single-cell RNA sequencing confirmed high cancer cell purity in early passages of LCOs derived from patients with advanced lung cancer. Furthermore, the three-dimensional structure and intracellular components of LCOs were characterized using three-dimensional holotomography. Finally, drug screening was performed using a specialized micropillar culture system with cryobiopsy-derived LCOs. LCOs derived from cryobiopsy specimens offer a promising solution to the critical limitations of conventional LCOs. Cryobiopsy can be applied to patients with lung cancer at all stages,

특허, PCT, 기술 이전, 사업화

출원 번호 통지서

출원 일자 2023.09.27
 특기사항 심사청구(유) 공개신청(무) 참조번호(P0923553)
 출원번호 10-2023-0130897 (접수번호 1-1-2023-1079612-72)
 (DAS접근코드E4AD)
 출원인 명칭 충남대학교병원(1-2016-076009-3)
 대리인 성명 김원준(9-1998-000674-0)
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 발명의 명칭 오가노이드 제작 방법

특 허 청 장

<< 안내 >>

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| | | |
|---------------|---|---------|
| 접수번호: | 612023007621718 | |
| 국제출원번호: | PCT/KR2023/015128 | |
| 접수일: | 27 September 2023 | |
| 수리관청: | Korean Intellectual Property Office | |
| 참조기호: | E23-0982CNUH | |
| 출원인: | 충남대학교병원 | |
| 출원인 수: | 1 | |
| 발명의 명칭: | 오가노이드 제작 방법 | |
| 제출된 문서: | E230982CNUH-appb.xml (E23-0982CNUH.xml) | 38019 |
| | E230982CNUH-dpcf-000003.zip | 3404300 |
| | E230982CNUH-fees.xml | 1785 |
| | E230982CNUH-poat-000001.xml | 2536 |
| | E230982CNUH-requ.xml | 9044 |
| | E230982CNUH-vlog.xml | 424 |
| 제출자: | Wonjoon KIM (고객 아이디: user_KR_KIM_WONJOON_5704) | |
| 접수 타임스탬프: | 27 September 2023 17:27 UTC+9 (KST) | |
| 제출에 관한 공식 요약: | 46:1D:B5:EF:BA:51:84:B4:BC:31:6C:DD:FD:08:F0:CF:6D:61:51:CC | |

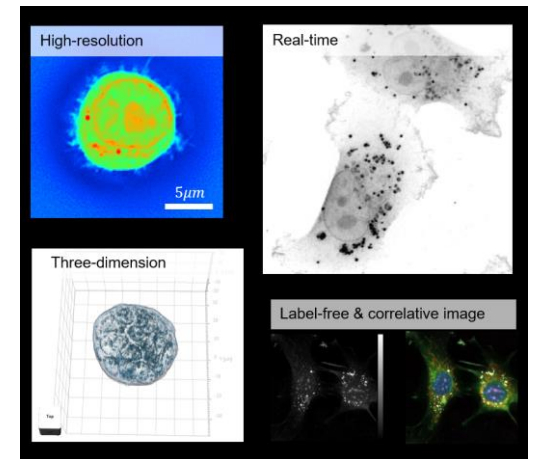
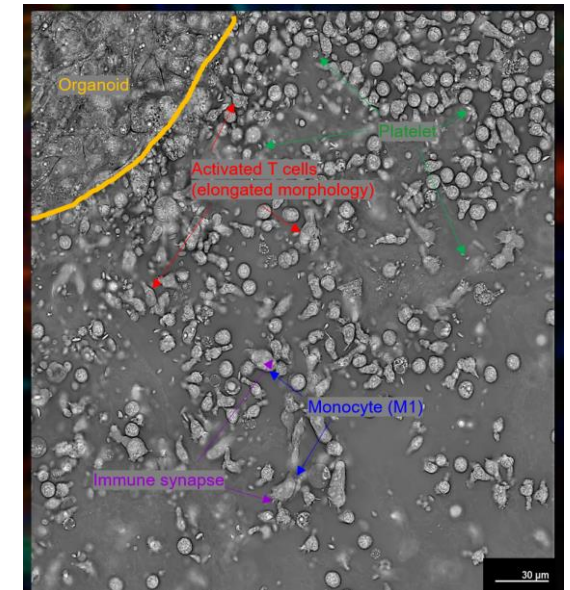
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오가노이드 심포지움, 기술 사업화



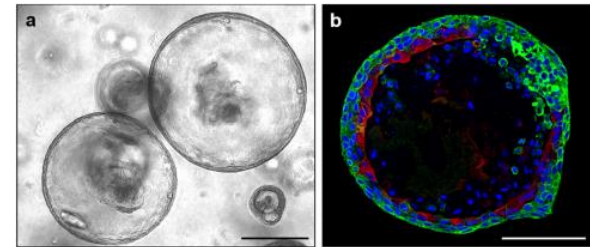
Future Research Plan

1. Establishment of a **Predictive Model for the Efficacy and Side Effects of Immune Checkpoint Inhibitors in Lung Cancer** by Co-culturing with Immune Cells Using Lung Cancer Organoids.
2. Development of a Deep Learning-Based Model Using Holotomography Imaging to **Differentiate between Lung Cancer Organoids and Normal Lung Organoids** and to Measure the Proportion of Cancer Cells.
3. Enhancing the Efficacy of Immunotherapy and Overcoming Resistance in Lung Cancer by **Modulating YAP and PD-L1 through Matrix Stiffness**

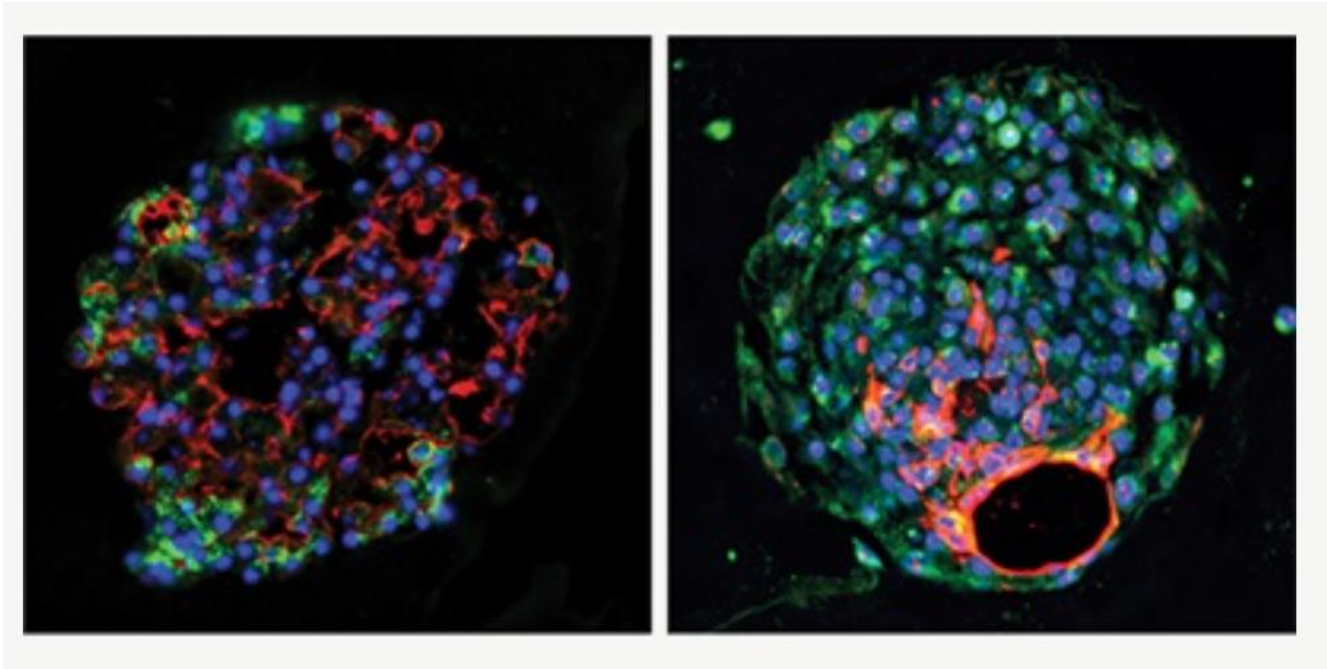


Take Home message : Check points in Organoid

1. What is the stem cell type and stem cell markers in this organoid?
2. What is the organoid formation efficiency in this model?
3. What are the media components in this organoid?
4. Is this organoid capable of long-term passage culture? And how long ?
5. Does this organoid grow well after thawing from cryopreservation?
6. Is this truly a cancer organoid?
7. What is the proportion of cancer cells in this organoid?
8. How can the purity of cancer cells be increased in this cancer organoid?



Determining how and where to employ organoids is of utmost importance



Organoid is an **ipad pro 12.9 inch with apple pencil.**



Thank you for your attention



Dongil Park



Dahye Lee

Acknowledgement

연구중심병원육성(R&D)사업



Cambridge University
Stem cell Institute
(Dr. Joo-Hyeon Lee)



GIST (Jinwook Choi)

감염 제어 컨버전스 연구센터
Infection Control Convergence
Medical Research Center (MRC)

KAIST (Jeong Seok Lee)
KAIST (Yong Ken Park)
KAIST (Jong Eun Park)



THANK
YOU!



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