

Single Cell (Transcriptomics) in COPD

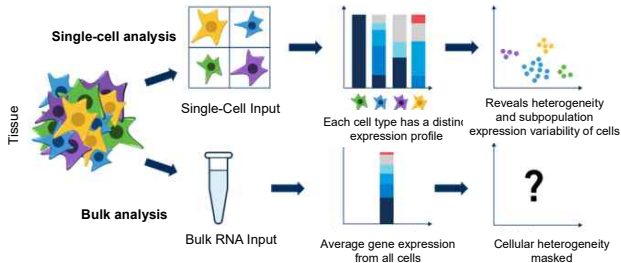
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Introduction to single cell analysis

Why Single Cells?



Single-cell RNAseq

Cell type A : 100 cells X mRNA 1 read
Cell type B: 1 cells X mRNA 100 reads

Bulk RNAseq

mRNA 201 reads from 101 cells
→ 2 reads per cell

What if that 1 cell matters most?

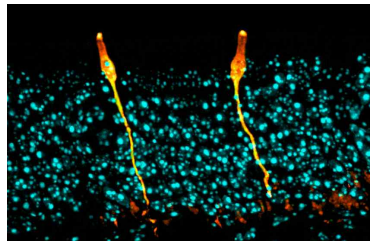
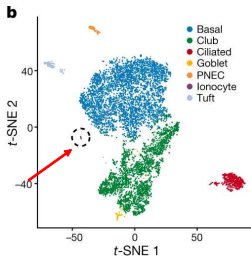
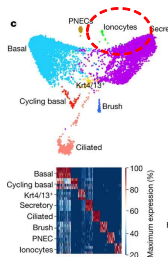
Ionocyte, rare but CFTR-dedicated cell

< 1% of airway epithelial cell

~190x more CFTR per cell than ciliated cells

~50% of all airway CFTR mRNA

CFTR loss → thick mucus → cystic fibrosis

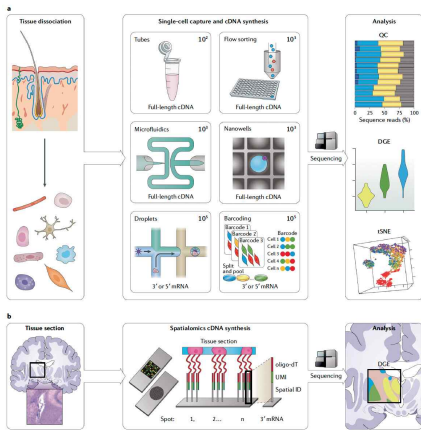


Plasschaert, Nature (2018)

Montoro, Nature (2018)

Lei, JCI (2023)

How to Capture Single Cells?



FACS

Microfluidics

Nanowells

Droplets

Combinatorial indexing

“Cell Barcoding” to cDNA → sequencing

How to Barcode?

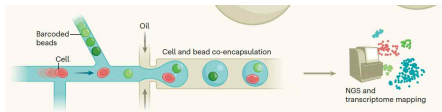
Droplet-based

Cell + barcoded bead → trapped in one oil droplet (GEM)

One droplet = one cell = one barcode

e.g. **10x Chromium**, inDrop, Drop-seq

Fast, scalable



Microwell

One cell sorted into one well (each well = one barcode)

e.g. plate-based SMART-seq (full-length), Seq-Well, BD Rhapsody
full-length, sensitive

Combinatorial indexing (split-pool)

No physical isolation - cells split & pooled across plates repeatedly

Each round adds a barcode → unique combination per cell

e.g. sci-RNA-seq, SPLiT-seq

no special device, very large scale

Sample preparation

Fresh (live-cell dissociation)

Highest RNA detection

Must process immediately

Dissociation stress, selection bias (fragile cells)

Cryopreservation (using freezing media)

High RNA detection

Longer storage

Dissociation stress, selection bias (fragile cell)

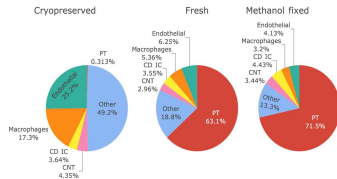
Snap-frozen → snRNA-seq

Lower RNA contents (nuclear RNA only)

Longest storage

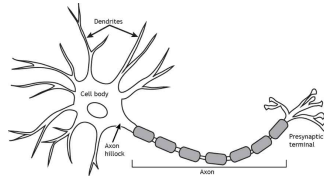
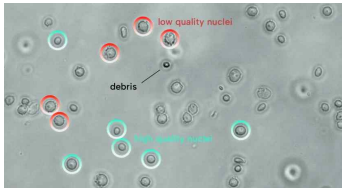
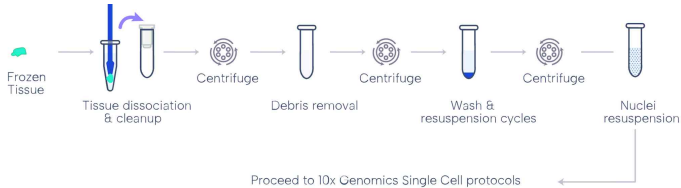
No dissociation stress

Fewer lymphocytes (under-represented)



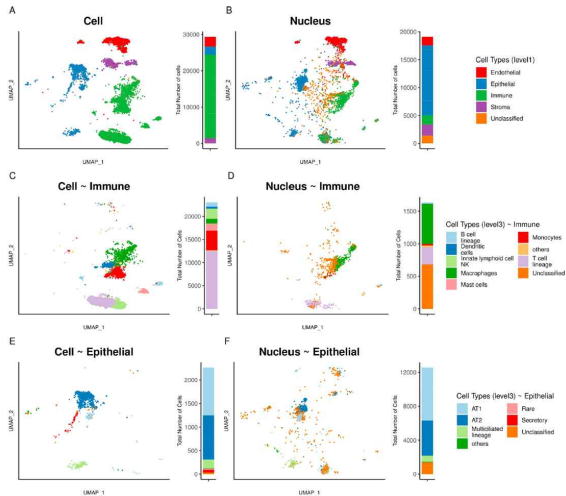
Cryopreservation can deplete epithelial cells (in kidney)

Single Nuclei RNA Sequencing (snRNAseq)



Best for fragile cells: neurons, muscle, and AT1

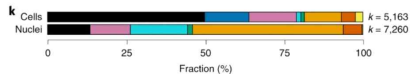
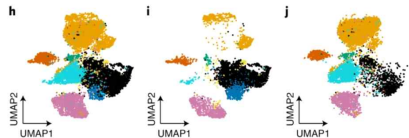
scRNAseq vs snRNAseq



Metastatic breast cancer (*HTAPP-963-SMP-4741*)

Cell type signature:

● B ● Endothelial ● Epithelial ● Fibroblast ● Hepatocyte ● Macrophage ● NK ● T



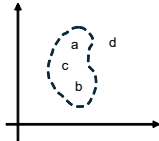
More immune cells in scRNAseq
More epithelial cells in snRNAseq

Finer subtype resolution in scRNAseq
More "unclassified" in snRNAseq

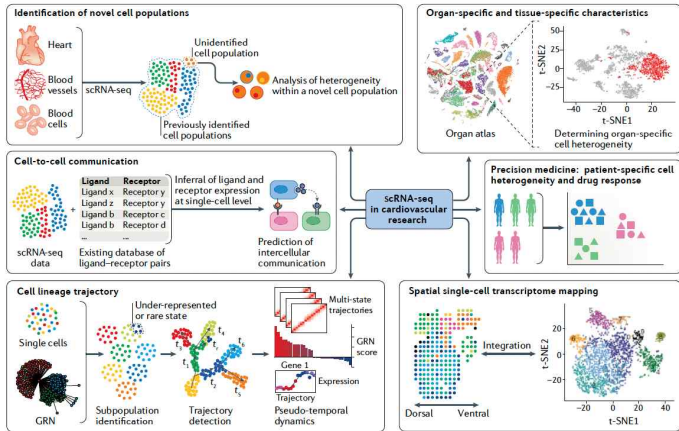
Applications of scRNA seq

Gene	Cell ID	a	b	c	d	...
X		4	0	0	15	...
Y		0	5	0	0	...
Z		1	0	7	2	...
.	
.	
.	

[Count matrix]



[Embedding]
[Clustering]

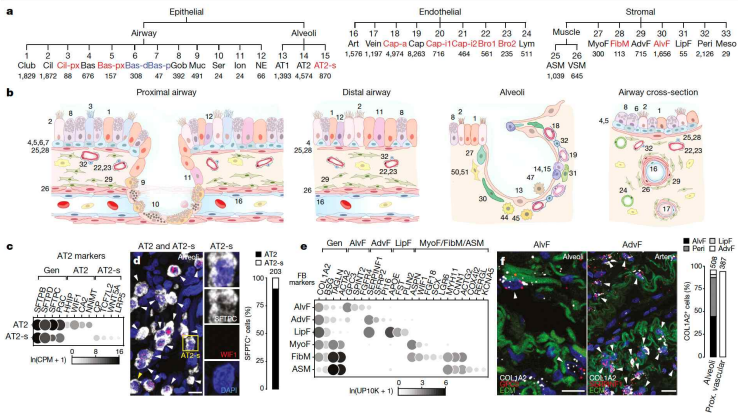


scRNA studies in COPD

- Epithelial cells

Single Cell Atlas of Human Lung

Normal (n=3)
Droplet/microwell
~75,000 cells



Defined 58 cell types

- 14 novel cell types

Histologic cell types

→ Molecular cell types

Examples

- Alveolar type 2 (AT2)

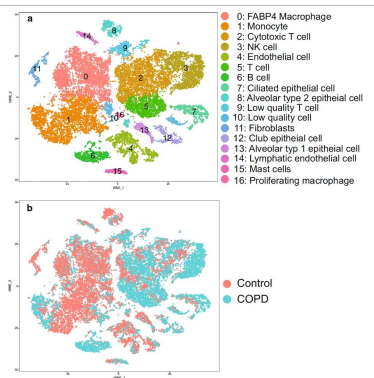
: AT2 vs AT2s

- Fibroblasts

: AlvF, AdvF, LipF, MyoF

COPD (n=3), normal (n=3)
severe COPD (explanted)
scRNAseq
29,961 cells

Different Gene Expressions of Cells in COPD



No significant cell composition shift (n=3)

DEGs shows T cell activation, ↑fibrinogens & antigen presentation

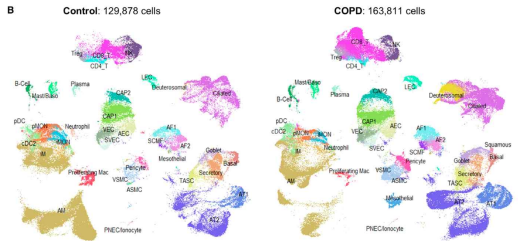
Cluster	Population	# DE genes	Up	Down	Control % of cells	COPD % of cells	Fold change	p value (t.test)	p value (Wilcox)
0	FABP4 Macrophages	868	620	248	27.97 ± 10.74	5.15 ± 1.57	5.43	0.02	0.057
1	Monocytes	1499	677	822	23.80 ± 12.90	11.24 ± 4.84	2.12	0.18	0.23
2	Cytotoxic T cells	539	165	374	11.18 ± 6.48	21.86 ± 9.80	-1.96	0.14	0.11
3	NK cells	431	233	198	3.85 ± 2.89	22.14 ± 27.41	-5.75	0.23	0.4
4	Endothelial	242	185	57	9.17 ± 3.90	2.37 ± 0.52	3.87	0.03	0.057
5	T cells	213	64	149	2.11 ± 1.00	11.68 ± 9.02	-5.54	0.08	0.23
6	B cells	153	30	123	0.99 ± 0.59	6.80 ± 7.95	-6.84	0.19	0.23
7	Ciliated	590	433	157	1.84 ± 0.98	6.18 ± 4.36	-3.35	0.10	0.23
8	AT2	476	34	442	4.12 ± 2.52	0.96 ± 0.32	-4.28	0.09	0.057
9	Low quality T cells	41	14	27	1.75 ± 0.68	3.71 ± 2.07	-2.12	0.13	0.11
10	Low quality cells	596	369	227	2.02 ± 0.95	2.83 ± 1.50	-1.40	0.41	0.63
11	Fibroblasts	96	87	9	3.17 ± 1.62	0.27 ± 0.07	11.60	0.03	0.057
12	Club cells	98	70	28	1.35 ± 0.81	2.72 ± 2.16	-2.02	0.29	0.63
13	AT1	68	59	9	2.60 ± 1.91	0.46 ± 0.36	5.71	0.12	0.11
14	Lymphatic-endothelial	27	20	7	1.93 ± 1.10	0.33 ± 0.29	5.85	0.06	0.23
15	Mast cells	32	25	7	1.32 ± 0.79	0.91 ± 0.24	1.44	0.44	0.63
16	Proliferative macrophages	111	55	56	0.84 ± 0.35	0.39 ± 0.25	2.17	0.12	0.11

COPD (n=5)
 scRNAseq
 55,417 epithelial cells (sorted)

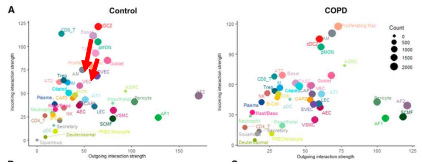
Progenitor Niche Dysfunction in COPD

A

	This Study	GSE196341		GSE168191		GSE178360
	COPD	COPD	Control	COPD	Control	Control
# donors	6	12	10	4	5	3
# cells	55,418	52,635	39,871	55,758	61,386	28,621
% of COPD cells or control cells	34%	32%	31%	34%	47%	22%



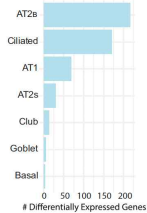
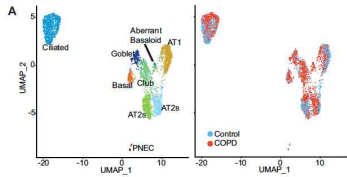
CellChat



Basal cell, TASC
 : decreased incoming signals

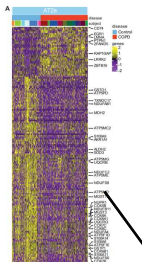
Aberrant Alveolar Niche in COPD

COPD (n=17), normal (n=15)
severe COPD (explanted)
scRNAseq
111,540 cells



AT2_B enriched for COPD GWAS genes
(HHIP, SERPINA1, SFTPD)
→ most COPD vs normal DEGs

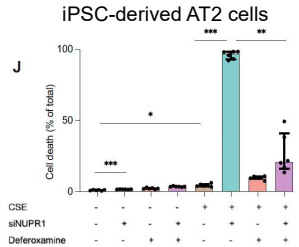
AT2_S: progenitor cell
AT2_B: surfactant producing cell



Up:
Oxidative stress / apoptosis

Down:
Electron transport chain
Antioxidant

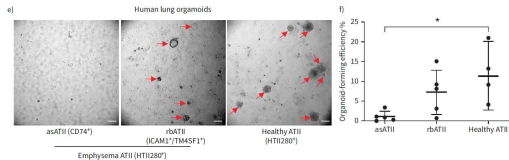
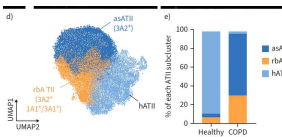
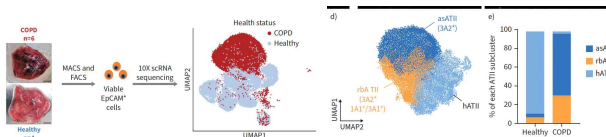
NUPR1: cellular stress response TF



→ Ferroptosis

Emphysema-Specific AT2 cells in COPD

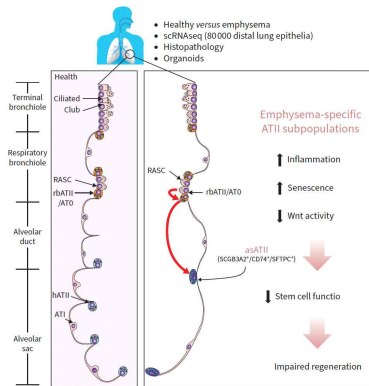
COPD (n=6), healthy (n=4)
GOLD stage 4 (explanted)
scRNAseq
78,699 epithelial cells (sorted)



Loss of normal AT2

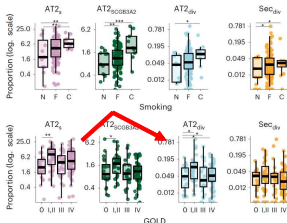
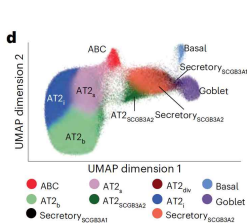
↑ asAT2/rbAT2 (emphysema-specific)

→ Lower stem cell function/ impaired regeneration



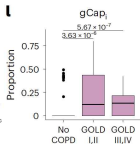
COPD(n=113), normal (n=28)
 Never smoker/smoker, GOLD 1~4
 Single-cell RNAseq
 1,516,727 nuclei

Regeneration to Maladaptation in COPD Progression

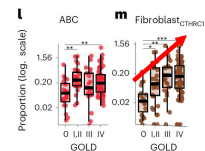
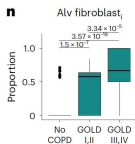
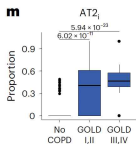


[Regenerative cell types]

Group	n	FEV ₁ % predicted (IQR)
Never-smoker	13	87 (16)
Normal spirometry	15	96 (9.5)
GOLD stages I, II	29	67 (20)
GOLD stage III	25	36 (9)
GOLD stage IV	59	20 (7.67)



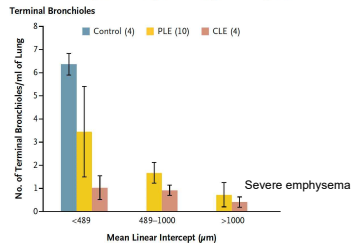
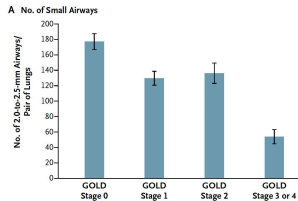
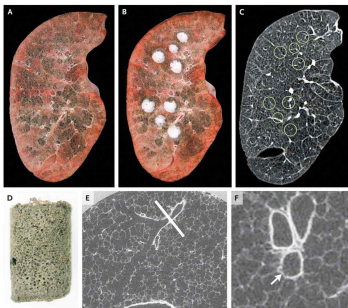
[Inflamed cell types]



[Maladaptive cell types]

ABC (aberrant basal cell)
 CTHRC1+ fibroblast
 → fibrosis related cell types

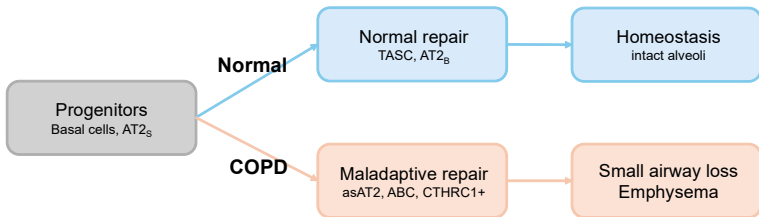
Small-airway Obstruction and Emphysema



Summary and Future Directions

Summary

- Smoking → progenitor loss/alteration in bronchioles and alveoli (TASC, AT2)
- Impaired regeneration → Maladaptive differentiation or progenitors
→ small airway loss and emphysema



Future Directions

- Nomenclature of cell types
 - Different names (TASC/RASC, AT0/AT2_{SCGB3A2})
 - Cell types vs cell states (AT2_B, AT2_S, AT2_i, AT0)
- Early COPD: Pre-COPD, PRISm
- Heterogeneity of COPD: COPD-Tbc, COPD-BE, COPD-ACO, etc.
- Response to treatments

- Well-annotated clinical data
- Clear questions → better design
- Validation: organoids, animal models
- Re-analysis of public datasets