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Cellular organization and biology of the respiratory system

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The lungs, which evolved in early terrestrial vertebrates, mediate the vital processes of supplying O_2 to the blood and removing CO_2. Air enters through the mouth and trachea and is distributed through the lung lobes by a highly branched, tree-like system of tubes lined by pseudostratified epithelium. The intralobar airways supported by cartilage are known as bronchi; the smaller branches lacking cartilage are known as bronchioles. The bronchioles terminate in millions of tiny, thin-walled, highly vascularized sacs (alveoli) composed of specialized epithelial cells where gas exchange takes place. In addition to epithelial cells, the respiratory tract contains various mesenchymal cell types, including smooth muscle and fibroblasts that support epithelial homeostasis and lung functions as well as ectoderm-derived neurons. Although respiratory tract tissue shows limited turnover in homeostasis, damage to the respiratory epithelium can be repaired by resident tissue stem cells. Furthermore, immune cells within the lung help protect the lungs from infection and promote repair. Defects in the homeostatic and reparative mechanisms may lead to diseases such as chronic obstructive pulmonary disease, asthma, fibrosis and cancer, which together make a major contribution to mortality worldwide.

Tissue organization and cell function

Airways

Basal cells: TP63, KRT5, NGFR
Function as multipotent stem cells

Club cells: SCGB1A1, SCGB3A2 (data from mice)
Immunomodulatory functions

Goblet cells: MUC5AC, FOXA3, SPDEF
Secrete mucins

Ciliated cells: FOXJ1, β-tubulin IV
Remove mucus out of the lung

NE cells: ASCL1, CALCA
Act as sensory cells; communicate with neurons

Rare cells
Ionocytes: FOXI1, CFTR high
Tuft (brush) cells: TRPM5, GNG13

SMGs

Myoepithelial cells: TP63, ACTA2
Multipotent stem cells; expel mucins out by contraction

Acinar cells
Serous: LTF, DCPP1
Mucous: MUC5B, TFF2

Alveoli

AEC2s: SFTPC, DC-LAMP
Stem cells; produce surfactant

AEC1s: PDPN, AGER
Large surface area; facilitate gas exchange

Mesenchymal cells

Smooth muscle cells: ACTA2, MYH11

Peribronchial fibroblasts: GLI1, LGR6
Myofibroblasts: ACTA2

AEC2 associated fibroblasts (lipofibroblast-like): PDGFRA

Pericytes: PDGFRB

Immune cells

Dendritic cells

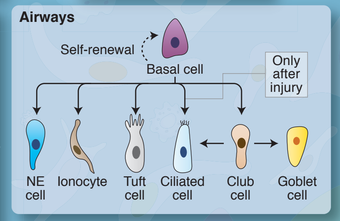
Alveolar resident macrophages, interstitial macrophages

Basophils, eosinophils

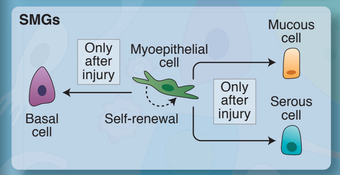
Lymphoid cells (T and B cells)

Cell lineages

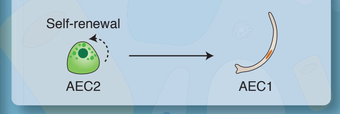
Airways



SMGs

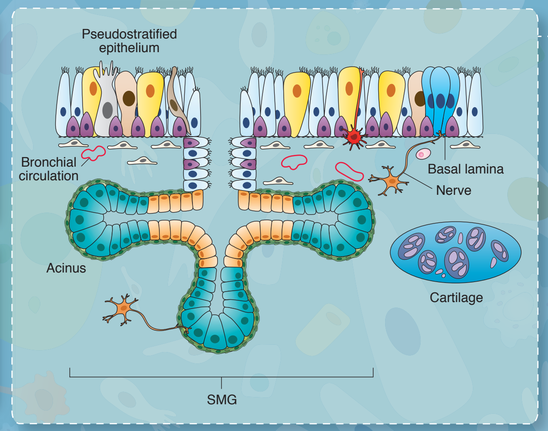


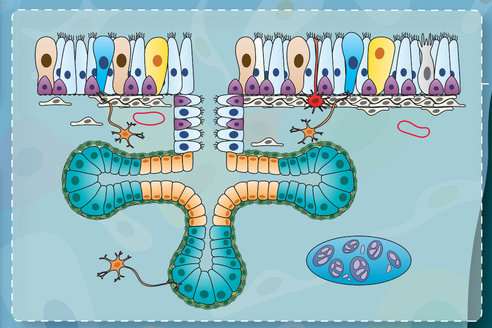
Alveoli



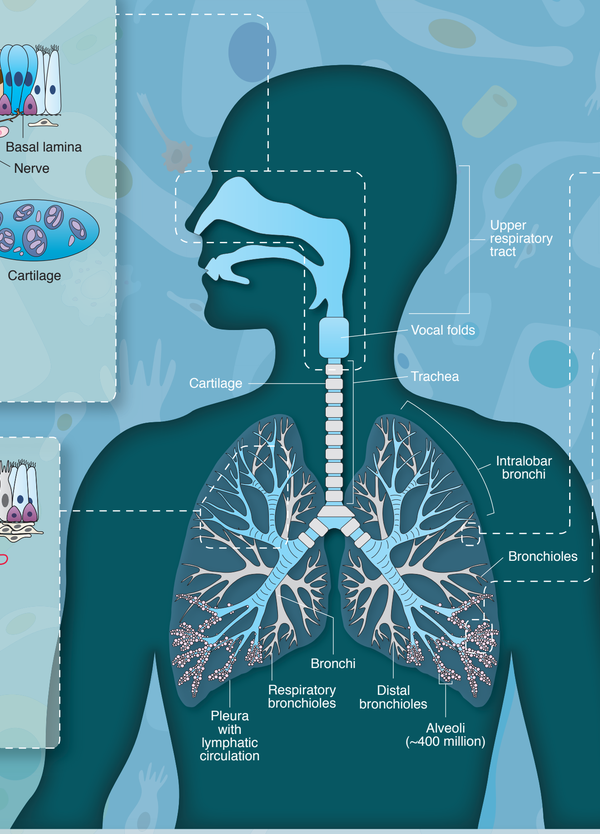
Mouse–human differences

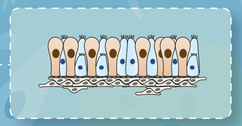
- Lung tidal volume is much smaller in mouse
- Goblet cells are less abundant in mouse
- SMGs are present only in upper-most trachea in mouse
- NE cells are mostly solitary and scattered in human airways
- Cartilage and basal cells are absent from mouse intralobar airways
- Respiratory bronchioles are absent from mouse airways

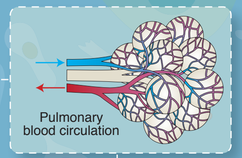


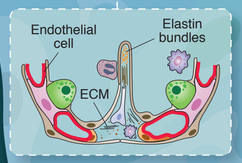


Human respiratory system









Respiratory diseases

Asthma

- Obstruction of small airways by goblet cell hyperplasia and excessive mucus production
- Constriction of small airways and reduced air flow caused by hypersensitive smooth muscles and tissue fibrosis
- Inflammation due to accumulation of eosinophils and other immune cells in airways
- Risk factors: allergens, environmental pollutants, obesity, maternal–fetal exposure, family history

Bronchopulmonary dysplasia

- Delayed development of alveolar region
- NE cell hyperplasia, inflammation
- Risk factors: premature birth, maternal exposures

Bronchiolitis

- Inflammation in small conducting airways
- Risk factors: viral infection, connective tissue disease, lung transplant rejection, burn-pit exposure, diacetyl exposure (rare)

Chronic obstructive pulmonary disease

Heterogeneous disorder including:

Emphysema:
- Abnormal, permanent enlargement of alveoli; reduced surface area for gas exchange
- Loss of alveolar septa and reduced lung elasticity
- Risk factors: smoking, mutations (loss of function of *SERPINA*)

Chronic bronchitis:
- Inflammation of mucosa in large airways, increased mucus production, cough
- Risk factors: smoking, inhalational exposures, infection, gastroesophageal reflux

Cystic fibrosis

- Reduced airway surface fluid and thickened mucus inhibit mucociliary clearance and promote airway colonization by microbes
- Risk factors: mutations (loss of function of *CFTR*)

Idiopathic pulmonary fibrosis

- Progressive, irreversible replacement of alveoli with scar-like deposits of ECM, containing hyperplastic AEC2s, cysts of bronchiolar epithelium and inflammatory cells
- Risk factors: advanced age, male sex, smoking, mutations (loss of function of telomerase genes (*TERT*, *TR*), surfactant proteins, and polymorphisms in MUC5B gene regulatory elements)

Pulmonary arterial hypertension

- Expansion of the tunica media in arterioles, endothelial plexiform lesions
- Risk factors: mutations (*BMPR2*, *ALK1*), drug toxicity (fen-phen), HIV, connective tissue disease

Lung cancer

Major types of lung cancers include:

ADCs (40% of cases):
- Originate from AEC2s with five subtypes differing in morphology and clinical characteristics
- Risk factors: mutations (gain of function: *KRAS*, *EGFR*; loss of function: *CDK2A*, *KEAP1*, *STK11*)

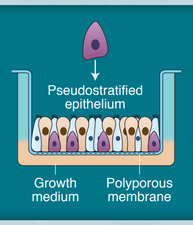
SCCs (30% of cases):
- Originate from basal cells and are characterized by stratified layers of flat, thin squamous cells
- Risk factors: smoking, mutations (*TP53*, *SOX3*, *TP63*, *PI3KCA*, *CDK2A*, and *FGFR1*)

SCLCs (15% of cases):
- Originate from neuroendocrine cells and are one of the most aggressive cancer types in humans
- Risk factors: smoking, mutations (*RB1*, *TP53*)

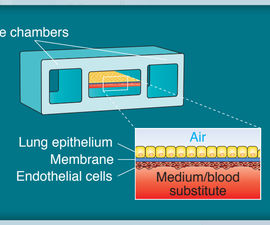
Lung cell culture models

- Use primary cells isolated from donor lungs after proteolytic digestion
- Undifferentiated basal cells, known as HBEs, and AEC2s can be cultured in 2D and 3D modules
- Used to identify growth and differentiation factors and screen for drugs to treat respiratory diseases
- 2D cultures also allow measurement of transepithelial resistance (and hence, evaluate epithelial integrity)
- 3D cultures are amenable for high-throughput screens
- 2D and 3D cultures allow measurement of ion channel activity (e.g., CFTR)

2D cultures

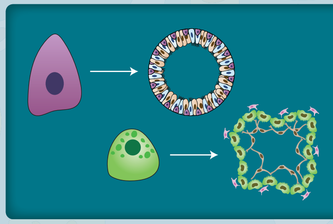


Air–liquid interface
- HBECs are grown on transwell inserts in specialized cell culture medium
- After reaching confluence, the liquid is removed to expose cells to air and the medium is changed to induce differentiation
- By 2–4 weeks, pseudostratified epithelium is formed



Lung on a chip
- A microfluidic-based assembly in which lung epithelial cells are grown on one side of a membrane and stromal cells on the other surface
- Liquid and air are circulated through the system to mimic air and blood flow in the lung

3D cultures (organoids)



- HBECs are seeded into ECM gels and generate spheres with basal, ciliated and secretory cells
- AEC2s are seeded into ECM gels with fibroblasts and/or endothelial cells
- In mouse-derived cultures, both AEC2s and AEC1s are present in these organoids; the efficiency of generating AEC1s from human AEC2s is currently limited

Abbreviations
ADC, adenocarcinoma; AEC, airway epithelial cell; CFTR, cystic fibrosis transmembrane conductance regulator; ECM, extracellular matrix; EGF, epidermal growth factor; HBECs, human bronchial epithelial cells; NE, neuroendocrine; SCC, squamous cell carcinoma; SCLC, small-cell lung carcinoma; SMG, submucosal gland

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