Mechanical tension on alveolar stem cells

qBIO
01/29/2021
Mechanosensing in the lung

Defective alveolar stem cells and mechanical tension lead to spatially specific fibrosis
Lung regeneration*: Pneumonectomy (PNX) as a model of compensatory growth

1) Unilateral removal of 1 lobe
2) Growth of remaining lobe

Which cells act as progenitor cells?
What signals are important to activate progenitors?
Alveolar Epithelial Cells: AT1 and AT2
Alveolar Epithelial Cells: AT2 Progenitor Function
Cdc42 signaling cascade is required for AT2 regenerative response to mechanical tension

MAPK-Mediated YAP Activation Controls Mechanical-Tension-Induced Pulmonary Alveolar Regeneration
Cell Reports, 2016
Mechanical tension induced AT2 signaling can lead to regeneration or progressive pulmonary fibrosis.
Is Cdc42 required for AT2 cell mechanosensing?

Figure 1

A

Sftpc-CreER;Rosa26-mTmG

dissociate lungs & FACS GFP+ cells

or

cultured for 3 days

analysis

non-stretched stretched

B

non-stretched stretched

GFP/GTP-Cdc42

C

GTP-Cdc42

fluorescence intensity

****

0 1000 2000 3000

0 1 2 3 4

non-stretched stretched

D

relative GTP-CDC42

in lung lysates

0 5 7 21

post-PNX days

Activation of Cdc42 in stretched AT2 cells and after PNX
Is Cdc42 required for AT2 cell differentiation to AT1 cell?

**Lineage tracing strategy**
Is Cdc42 required for AT2 cell differentiation to AT1 cell?

Figure 1

Cdc42 required for AT2 regeneration of alveolar compartment after PNX
Is Cdc42 required for AT2 cell differentiation to AT1 cell?

Cdc42 required for AT2 regeneration of alveolar compartment after PNX
AT2 progenitor function prevents alveolar simplification after PNX or with aging
What are the long-term consequences of failed AT2 regeneration following PNX?

Decreased lung function and progression of outside-in fibrosis
What cell types in the lung mediate fibrosis following PNX when AT2 cell regeneration is compromised?

Figure 2

Mesenchymal myofibroblast proliferation contributes to fibrotic phenotype
Figure 3

There are 2 populations of AT2 cells; subpopulation I is increased in the mutant following PNX.
What are the molecular characteristics of subpopulation I AT2 cells?

> 2/3 of upregulated genes in subpopulation I are also upregulated in IPF
Is increased mechanical tension responsible for the progression of lung fibrosis?

Figure 4

Increased tissue stiffness at the periphery during outside-in lung fibrosis
Is increased mechanical tension responsible for the progression of lung fibrosis?

Reducing mechanical tension leads to rescue of fibrotic phenotype
Figure 5

What signaling pathways mediate mechanical tension induced fibrosis?

Stretched AT2 cells express Tgf-B locally
What signaling pathways mediate mechanical tension induced fibrosis?

**Figure 5**

**D**

mouse

GFP/p-SMAD2/DAPI

- non-stretched
- stretched
- stretched + anti-TGFβ
- stretched + GSK3008348

**E**

P- SMAD2+ AT2 cells (%)

- stretch
- anti-TGFβ
- GSK3008348

- **F**

human

HT1-2006β-SMAD2/DAPI

- non-stretched
- stretched
- stretched + anti-TGFβ
- stretched + GSK3008348

**G**

P- SMAD2+ AT2 cells (%)

- stretch
- anti-TGFβ
- GSK3008348

Downstream target of Tgf-B signaling upregulated in AT2 cells
Is mechanical tension spatially regulated within the lung?

Peripheral alveoli are more stretched at high inflation pressure.
What signaling pathways mediate mechanical tension induced fibrosis?

Figure 6

Tgf-B signaling is activated at the periphery at high inflation pressure
What signaling pathways mediate mechanical tension induced fibrosis?

Figure 6

Tgf-B signaling in AT2 cells is activated in fibrosis model
Is Tgf-B signaling required for progression of lung fibrosis?

Loss of Tgf-B signaling rescues fibrotic phenotype due to failed alveolar regeneration following PNX
Mechanical tension induces AT2 signaling can lead to regeneration or progressive pulmonary fibrosis.
Discussion Questions

• What niche signals are important for AT2 stem cell response to mechanical tension?
• What’s the signal from AT2 cells to fibrotic cells?
• What regenerative pathways are important to protect the lung from fibrosis during aging?
• Why does AT2 regenerative ability decrease with age?
• Are there treatments that can reduce mechanical stress and prevent fibrosis?
Questions about lung regeneration/repair?

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Airway Smooth Muscle Cells in Lung Branching
Airway smooth muscle surrounds the airway epithelium
Prevailing hypothesis: Airway smooth muscle promotes lung branching

Brennan et al. PLOS One 2013
Chemical inhibition of smooth muscle contraction disrupts epithelial branching ex vivo


Goodwin and Nelson et al. Development 2019
In vivo testing of the prevailing hypothesis

Airway Smooth Muscle

\[\rightarrow\]

Airway Smooth Muscle Contraction

\[\rightarrow\]

Airway Peristalsis

\[\rightarrow\]

Airway Pressure

\[\rightarrow\]

Lung Branching
Myocd^{CKO} inactivation led to loss of airway smooth muscle

**ACTA2**

- **Control**
- **Tbx4rtTA;tetOcre;Myocd^{FrF}**

**CDH1**

- **E12.5**

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<th>Control</th>
<th>Myocd^{CKO}</th>
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<td>Acta2</td>
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Relative mRNA Abundance

- ***p < 0.0005, **p = 0.005, *p < 0.05
- N = 3 for each control and Myocd^{CKO} group
Inhibiting airway smooth muscle differentiation does not disrupt lung epithelial branching
Inhibiting airway smooth muscle differentiation does not disrupt lung epithelial branching.
Airway smooth muscle is dispensable for lung branching morphogenesis in vivo

![Diagram showing the relationship between airway smooth muscle and lung branching](image-url)