Cells and Maturation of the Lung

CHIR Group in Lung Development
Hospital for Sick Children, Toronto

5th World Congress on Pediatric Critical Care, Geneva
Congenital malformations
Congenital Diaphragmatic Hernia
Congenital Diaphragmatic Hernia

Lung Characteristics

- Hypoplasia
- Reduced airway branching
- Diminished vascularization
- Reduced alveolar number
Premature birth
Intrauterine inflammation
Ventilation/oxygen
Prematurity

- 5-10% of all births
- 75% of neonatal deaths
- 85% of all neonatal complications
Consequence: Bronchopulmonary Dysplasia

Lung Characteristics

Born: 26 wk; Biopsy: 7 mth
Requirements of Lung Development

- Growth
- Differentiation
- Morphogenesis
Endodermal Lung Specification

Factors

Foxf2a, TTF-1, GATA-6, Gli2 and Gli3, RA/RAR

foregut

trachea
Tracheal Outgrowth and Bronchial Bud Formation

Human

Mouse

Transcription Factors
Nkx2.1(TTF-1)
Hoxa5

Growth Factors
FGF10/FGFR2-IIIb
Pulmonary Branching

(Conducting airways)

Question: What guides branching morphogenesis?
MESENCHYMAL-EPITHELIAL INTERACTIONS

- Removal of mesenchyme
Bud branching simplified
FOXA transcription factors regulate SHH required for branching morphogenesis and smooth muscle cell differentiation

Maeda, Y. et al. Physiol. Rev. 2007
How does vascular system develop?
Development of the pulmonary vessels

From the aortic sac a vessel plexus arises around the lung anlagen that with the lung buds extends caudally (32 days)

1. First aortic arch
2. Second aortic arch
3. Third aortic arch
4. Fourth aortic arch
5. Dorsal aorta
6. Lung buds
7. Aortic sac
8. Pulmonary plexus
Vascular development of the lung

- **Angiogenesis**: the branching of new vessels from preexisting ones → central

- **Vasculogenesis**: the development of blood lakes that transform into vessels → peripheral
Lung vascular morphogenesis models

A

MODEL 1

MODEL 2

MODEL 3

Distal vasculogenesis

Distal angiogenesis

B

"Tip zone"

Capillary plexus

Vein

Artery

Airway muscularization level

Artery muscularization level

BA

BA.1

BA.2

BA

Capillary plexus

Vascularization: In Utero Environment

Intervillous space opens around 10-12 wks gestation

\[ pO_2: 15-20 \text{ mM Hg} < 10-12 \text{ wks} > pO_2: 55 \text{ mm Hg} \]

Ductus arteriosus shunts 90% of blood from the fetal lung

Consequence: Fetal lung develops in relative Low Oxygen environment
Low Oxygen Tension Promotes Vascular Development

(D11 lung explants of Tie-LacZ mice)

48h

72h

20% 3%

Oxygen concentration
Oxygen-sensing via HIF-1α protein
(Semenza: PNAS, 99:11570-11572, 2002)
Stabilization of HIF-1 with DMOG Stimulates Vessel formation

A

B

Control  DMSO  500 μM DMOG  1000 μM DMOG
EFFECT OF INHIBITION OF VEGF SIGNALING ON EARLY LUNG DEVELOPMENT

Control

VEGFR2 inhibitor
SU5416
Treatment of newborn rats with a VEGF receptor inhibitor
(barium angiograms)

A
Control
SU5416

infant rats

B
Control
SU5416

adult rats

Alveolar Morphogenesis

1. Type I cell
2. Airsac
3. Type II cell
4. Basal lamina of airsac
5. Basal lamina of vessel
6. Endothelium of the vessel
Alveolarization

Processes involved

1. Cell growth
2. Septation
3. Microvascularization
4. Apoptosis
I. Elastin deposition in primary septa

II. New secondary septa with double capillary layer

III. Microvascularization with fusion of capillary layer in single medial layer and thinning of interstitium

- Increased MMP-2/-9 activity

- Elastin/decorin/CS at the tips

- PDGF-αR pos myofibroblasts

- Elastin/Tenascin-C

- Capillary
Alveolarization

Processes involved

1. Cell growth
2. Septation
3. Microvascularization
4. Apoptosis

Question ?
Epithelial Overexpression of Oxygen Insensitive HIF-1α

Does overexpression postnatally affect VEGF expression, vessel formation and alveolar formation?
Postnatal Lung histology of C57 wild-type pups and HIF-1α ΔODD transgenic pups

C57 WT

HIF-1α ΔODD

Postnatal days
Overexpression of HIF-1α ΔODD Increases Postnatal Peripheral Vessel Number

Vessel Density

Vessels (60-120 mm) / mm²

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<th>C57</th>
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* indicates statistical significance.
Overexpression of HIF-1α ΔODD Increases Postnatal Septal Formation and Alveolar Number
Improved lung growth and function through hypoxia-inducible factor in primate chronic lung disease of prematurity


Bronchopulmonary dysplasia (BPD), a chronic lung disease affecting preterm neonates, is associated with significant childhood and adult health problems. Histopathologic features of BPD include impaired vascular and distal airway development. We previously showed that activation of hypoxia-inducible factors (HIFs) by inhibition of prolyl hydroxylase domain-containing proteins (PHDs) is feasible and that it stimulates vascular endothelial growth factor (VEGF)-dependent angiogenesis in vitro. We tested the hypothesis that enhancement of angiogenesis by activation of HIFs improves lung growth and function in prematurely born neonates in vivo. Preterm baboons (125 day+14 day pre nata O2 model, corresponding to 27 human gestational weeks) were treated for 14 days with intravenous (i.v.) FG-4095, a PHD inhibitor. Notably, 77% of diminished total alveolar surface area in untreated controls was recovered by FG-4095 treatment. Functional significance of the structural changes was indicated by improved oxygenation and lung compliance in FG-4095-treated newborns. Surfactant proteins B and C and saturated phosphatidylcholine were unchanged. Incidence of spontaneous ductus arteriosus closure was increased, likely contributing to lower ratio of pulmonary to systemic blood flow in FG-4095 group. These findings indicate that HIF stimulation by PHD inhibition ameliorates pathological and physiological consequences of BPD.
Epithelial Differentiation

**Question:** Which pathway(s) regulate(s) epithelial cell patterning along the anterior-posterior axis in the lung?
Selective expression of transcription factors in the respiratory epithelium

Maeda, Y. et al. Physiol. Rev. 2007
TTF-1, FOXA2, NFATC3, and C/EBPα participate in a network regulating perinatal lung maturation and adaptation to airbreathing at birth.
Formation of the lung is dependent on a myriad of interactions of signaling and receiving molecules controlling proliferation and differentiation.

**Remaining Question:** How are the different pathways integrated and coordinated at the cellular and molecular level and can we build a lung in vitro?
Contributors

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Lung Tissue Stem Cells in Lung Regeneration

Bone-marrow stem cells

Type II cells

Type I cells

Clara cells

Ciliated cells

PNECs

Type II cells in Alveolar Epithelium

Clara cells in Bronchiolar Epithelium

NEBs at Bronchiolar Branch points

PNECs

Clara cells ?

Type II/I cells ?

Alveolus

Terminal Bronchiole

BV
Development of the pulmonary vessels (2)

Also from the dorsal aorta a vessel plexus forms that communicates with the ventral one and thus creates a connection between the ventral aortic sac and the dorsal aorta (36 days).

1. First aortic arch
2. Second aortic arch
3. Third aortic arch
4. Fourth aortic arch
5. Dorsal aorta
6. Lung buds
7. Aortic sac
8. Pulmonary plexus
Pregnant mice were injected at E9.5 with nitroimidazole hypoxia marker EF5 and analyzed at E10.5. Developing lung structures were visualized by EF5 antibody and red colour is indicative of an hypoxic environment.