Gestational Development of the lungs

Daphne E. deMello, M.D.

Faculty Disclosure Information

- I have no financial relationship with any manufacturer of any commercial product and/or provider of commercial services discussed in the CME activity.
- I do not intend to discuss an unapproved/investigative use of a commercial product or device in my presentation.
Objectives

• Learn the developmental processes that contribute to the formation of the lung’s airways, airspaces and vessels

• Understand the functional implications of disordered lung development

Laws of Lung Development

• **Law 1.** The bronchi and bronchioli develop by 16 weeks.

• **Law 2.** The alveoli multiply from $20 \times 10^6$ to $300 \times 10^6$ postnatally.

• **Law 3.** Preacinar arteries appear with the airways; intra-acinar arteries with the alveoli.
NEWBORN LUNG DISEASE

- DEVELOPMENTAL DISORDERS
  - Growth
  - Maturation
- ACQUIRED
  - Iatrogenic
  - Infection
- OTHER
  - Meconium aspiration
  - Massive hemorrhage
  - Chronic pneumonitis
  - Neuro-endocrine hyperplasia of infancy (NEHI)

Disorders of lung growth-
Airways/Air passages

- Hypoplasia/aplasia
- Bronchial atresia
- Acinar dysplasia
- Congenital pulmonary adenomatoid malformation (CPAM)
- Sequestration
- Congenital lobar emphysema (CLE)
Disorders of lung growth- Vessels

- Abnormal vascular growth – PPHN / MAS
- Alveolar capillary dysplasia (ACD)/ misalignment of vessels
- Congenital pulmonary lymphangiectasia

Disorders of lung maturation

- Prematurity – deficiency of surfactant lipid and protein (HMD & SP-A deficiency)
- Pulmonary interstitial glycogenosis (PIG)
- Congenital alveolar proteinosis
  - SP-B deficiency
  - SP-C deficiency
  - GM-CSF mutation
  - ABCA3 mutation
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Lung hypoplasia - causes

- Prolonged oligohydramnios
- Renal agenesis
- Renal cystic disease
- Obstruction of the lower urinary tract
- P.R.O.M.
- Skeletal dysplasia
Lung hypoplasia - analysis

- Lung weight < 50% N.E.W
- Lung weight:body weight  N= 0.012
- Reduced bronchial generations
- Decreased radial alveolar count
- Reduced alveolar number
- Reduced number of air-blood barriers
- Reduced number of intra-acinar arteries

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Clinical Summary

- Term boy with MCA and DNR status
- Dandy-Walker anomaly
- CDH – left lobe of liver, stomach, small bowel and spleen in left thoracic cavity
Disorders of lung growth-
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### Pathologic features- CPAM

<table>
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<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Frequency %</td>
<td></td>
<td>1-3</td>
<td>&gt;65</td>
<td>20-25</td>
<td>8</td>
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<tr>
<td>Cyst size cm</td>
<td>0.5</td>
<td>10</td>
<td>2.5</td>
<td>2</td>
<td>7</td>
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<td>Pathologic features</td>
<td></td>
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<tr>
<td>Bronchial septa</td>
<td>Present 100%</td>
<td>Present 33%</td>
<td>Absent</td>
<td>Absent</td>
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<tr>
<td>Fibrous septa</td>
<td>Bronchioles Solid</td>
<td>Alveolar lining</td>
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<tr>
<td>Solid</td>
<td></td>
<td></td>
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<tr>
<td>Alveolar lining</td>
<td></td>
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</table>

![Type I, Type II, Type III images]
Type 1 CPAM
Disorders of lung growth- Airways/Air passages

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PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN (PPHN)

- Precocious muscularization
  - genetic
  - stress
  - ductal constriction
- Pulmonary hypoplasia
- ↑ vasoactive agents
Clinical Summary

- 3 d old term boy, CS – TMC
- RDS & PPHN
- Transferred to PCH for ECMO
- Expired before ECMO could be initiated
Lung Barium Angiogram

Processes involved in vessel development

**ANGIOGENESIS** - the sprouting of vessels from preexisting ones

**VASCULOGENESIS** - the formation of vessels from “blood lakes”
Processes involved in vessel development

Noden – Am Rev Respir Dis. 1989
Structure – Function Relationship

Methods

Mercox

Right Ventricle

Pulmonary Vascular Cast

NORMAL LUNG
TEM – 9 DAYS

2,800 X 8,750 X

2,800 X 8,750 X
**Normal Lung**
TEM – 10 days

5,250 X

**Normal Lung**
Mercox Vascular Cast

12 days

13 days
Normal Lung
Mercox Vascular Cast

13 days

15 days
16 days
CONCLUSION

Early events in mouse lung vascular development

ANGIOGENESIS  VASCULOGENESIS

FUSION

CIRCULATION ESTABLISHED

[Images of historical figures]
Carnegie Collection of Human Embryos and Fetuses

housed in the

Human Developmental Anatomy Center

at the

National Museum of Health and Medicine

of the

Armed Forces Institute of Pathology, Washington, D.C.

Human Lung Vascular Development: Processes involved

Angiogenesis

Vasculogenesis

Fusion

Transcription Factors

Growth Factors and Receptors

Primitive Mesenchyme → Hemangio blasts → Endothelial Cell Commitment “Blood island” → Migration and Tube Formation → Smooth Muscle Recruitment and Differentiation → Mature Blood Vessel

Transcription Factors:
- SCL/tal-1
- Ets-1
- Vezf1
- ARNT
- ELF-1
- EPAS
- Fk-1
- GATA2
- GATA3
- HIF-1α
- HOXD1
- NERE2

Growth Factors and Receptors:
- bFGF
- Fk-1
- Flk-1
- Integrin αvβ3
- P/GH
- TGF-β
- TIE2
- VEGF
- Angiopoietin-1
- TIE2
The VEGF 120/120 mouse


Vascular Endothelial Growth Factor (VEGF)

• Best studied angiogenic growth factor

• Plays a critical role in stimulating vessel growth

• Deletion of even a single VEGF allele is lethal

• Three VEGF isoforms expressed in mouse lung
Distribution of VEGF Isoforms in Mouse lung

- VEGF 120 (31%)
- VEGF 164 (23%)
- VEGF 188 (46%)

Relative Amounts of VEGF Isoforms in Fetal Mouse Lung
VEGF Gene

WILD TYPE

3 4 5 6 7 8

VEGF 120

Exons 6 and 7 deleted

VEGF Gene

WILD TYPE

3 4 5 6 7 8

VEGF 120

Exons 6 and 7 deleted
Study Design

Swiss-Weber mouse fetuses (9-20 days gestation)

TEM of lung specimens
SEM of lung vascular casts

Study vessel developmental processes

VEGF Genotyping

BamHI

-9.5kb
-7.8kb
17-day-old VEGF 120 Transgenic Fetal Mouse Whole Body Vascular Casts

WILD TYPE

15-day-old VEGF120 Transgenic Fetal Mouse Lung Vascular Casts

WILD TYPE  HETEROZYGOUS  HOMOZYGOUS
SEM of 15-day VEGF 120 Transgenic Fetal Mouse Lung Vascular Casts

WILD TYPE          HETEROZYGOUS        HOMOZYGOUS

MAG X 43

SEM of 15-day VEGF 120 Transgenic Fetal Mouse Lung Vascular Casts

WILD TYPE          HETEROZYGOUS        HOMOZYGOUS

MAG X 700
17-day-old VEGF120 Transgenic Fetal Mouse Lung Vascular Casts

SEM of 17-day VEGF 120 Transgenic Fetal Mouse Lung Vascular Casts

WILD TYPE  HETEROZYGOUS  HOMOZYGOUS

WILD TYPE  HETEROZYGOUS  HOMOZYGOUS

MAG X 230
18-day-old VEGF120 Transgenic Fetal Mouse Lung Vascular Casts

- WILD TYPE
- HETEROZYGOUS
- HOMOZYGOUS

SEM of 18-day VEGF 120 Transgenic Fetal Mouse Lung Vascular Casts

- WILD TYPE
- HETEROZYGOUS
- HOMOZYGOUS

MAG X 140
1-day-old VEGF120 Transgenic Mouse Pup Lung Vascular Casts

SEM of Post Natal Day 1 VEGF 120 Transgenic Mouse Lung Vascular Casts
15-day-old Mouse Fetuses

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<tr>
<td>HOMOZYGOUS</td>
<td>0.0516*</td>
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<tr>
<td>HETEROZYGOUS</td>
<td>0.0858</td>
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<td>WILD TYPE</td>
<td>0.1168</td>
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*p<0.02

Post Natal Day 1

WT 120/120
### Postnatal 1-Day-Old Mouse Pups

<table>
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<th>Number of Air-Blood Barriers</th>
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<tr>
<td>HOMOZYGOUS</td>
<td>1.15*</td>
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<tr>
<td>HETEROZYGOUS</td>
<td>3.45</td>
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<td>WILD TYPE</td>
<td>4.65</td>
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*p<0.0001

### Conclusion

Absence of VEGF 164 and 188 isoforms:

- impairs the development of the lung’s microvasculature
- delays airspace structural maturation
Clinical Summary

- Infant born at 41wks gestation, meconium staining noted
- Hypoxemia, respiratory difficulty shortly after birth
- Requiring mechanical ventilation, NO, ECMO
- Death at two weeks of age
PPHN I - due to maldevelopment

Abnormal vascular *structure* possibly a reflection of aberrant angiogenesis

*Unlikely to respond to℞s*
PPHN II - due to maladaptation

Vascular *structure* normal, but absence of post-natal increase in pulmonary vascular compliance—*most likely to respond to Rs*

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**Clinical Summary**

- Infant born at 30 wks gestation
- Gasping respirations at birth
- Unable to ventilate
- Death in a few minutes
Anatomic Findings

- Hypoplasia of pre-and intra-acinar pulmonary arteries
- Precocious muscularization
- Thick media and adventitia
- Interstitial emphysema
Maternal Cocaine Use

PPHN III - due to hypoplastic pulmonary vascular tree

*Underdevelopment* of the pulmonary vasculature possibly a reflection of aberrant angiogenesis

*Unlikely to respond to Rs*
Disorders of lung growth- Vessels

- Abnormal vascular growth – PPHN / MAS
- Alveolar capillary dysplasia (ACD)/misalignment of vessels
- Congenital pulmonary lymphangiectasia

Clinical Summary

- Infant born at 36 wks gestation
- Hypoxemia and apnea shortly after birth
- Placed on ECMO for 2 wks
- Could not be weaned
- Death at 2 wks post-natal age
Alveolar Capillary Dysplasia
Functional Implications

- **Impaired angiogenesis:**
  - PPHN (III), hypoplastic vascular tree
  - Absence of PA
  - Misalignment of blood vessels

- **Impaired vasculogenesis:**
  - Alveolar capillary dysplasia
  - Angiomatosis

- **Abnormal fusion**
  - Arteriovenous malformation
Disorders of lung maturation

• Prematurity–deficiency of surfactant lipid and protein (HMD & SP-A deficiency)
• Pulmonary interstitial glycogenosis (PIG)
  • Congenital alveolar proteinosis
    - SP-B deficiency
    - SP-C deficiency
    - GM-CSF mutation
    - ABCA3 mutation

Pulmonary alveolar proteinosis

• Congenital AP
  SP-B deficiency
  SP-C deficiency
  ABCA3 Mutation
  GM-CSF/ GM-CSF 3/5 β- Receptor deficiency
• Acquired AP
Clinical Summary

- Full term newborn develops severe respiratory distress shortly after birth
- Treated with surfactant, $O_2$, ventilation, ECMO
- Lung biopsy
- Death shortly thereafter, no autopsy
SP-B deficiency - absent transcripts

SP-B deficiency
SP-B deficiency – index family

SP-B mutations

- 457delC (45delC)
- ATG
- 1549C/GAA (121ins2)
- 1553delT (122delT)
- G2417A (G135S)
- C3480T (R236C)
- Alternative splicing site
- TGA
- Abberent SP-B mRNA in CAP patient is split in intron 4
Thank you

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