Vascular-Epithelial Interactions During Lung Development: Clinical Implications

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DISCLOSURE STATEMENT

Steven Abman has disclosed the information listed below. Any real or apparent conflict of interest related to the content of his presentation has been resolved.

Role, Company:
• Grant recipient, Bayer HealthCare

Bronchopulmonary Dysplasia

BPD in the Post-Surfactant Era

“New BPD” (current)
“Old BPD” (Northway, 1967)
Evolution of the "New BPD"

"Classic BPD" (Marked Lung Injury)
- Extensive, diffuse fibroproliferation
- Alternating atelectasis with hyperinflation
- Severe airway epithelial disease and smooth muscle hyperplasia
- Decreased alveoli and abnormal vessels

"New BPD" (Arrest of distal lung development)
- Rare fibrosis
- Less regional heterogeneity
- Milder airway lesions
- Reduced alveolarization and dysmorphic vascular growth

Pathogenesis of BPD

- Genetic Factors
- Hyperoxia
- Inflammation
  - O₂⁻
- Disruption of growth factor signaling pathways
  - O₂⁻
- Premature Birth
- BPD
- O₂⁻
- Prenatal factors
  - Chorioamnionitis
  - Fetal infection
  - Smoking, drug use
- Infection
- Ventilator Induced Injury
- O₂⁻
  - Alveolarization
  - Vascular Growth
  - Lung Function

Persistence of Abnormal Structure of the Distal Lung in Late BPD
Late Cardiopulmonary Disease in a Young Adult with BPD

Late Pulmonary Hypertension is Associated with High Mortality in BPD (Khemani et al, Pediatrics, 2007)

Decreased endothelial NOS in the Fawn-Hooded Rat (Tyler et al, 1999)

Fawn Hooded Rats at Denver's Altitude Exhibit Lung Hypoplasia

(Fawn Hooded) Sprague Dawley

Fetal Adult
Chronic Pulmonary Hypertension Impairs Fetal Lung Growth

Pathogenesis of the “New” BPD

Prenatal Inflammation
- Chorioamnionitis
- Fetal Infection

Postnatal Lung Injury
- Oxygen (High/Low)
- Mechanical Ventilation
- Infection
- Inflammation
- Hemodynamics (PDA)

Premature Lung

Vascular Growth

Alveolarization

Chronic Lung Disease of Infancy

“BPD: A Vascular Hypothesis”

- Disruption of angiogenesis during critical periods of lung growth impairs alveolarization.
- Dysmorphic vascular growth and endothelial dysfunction increases the risk for pulmonary hypertension.

Antiangiogenesis Agents Decrease Alveolarization

(Jakkula et al. 2000)
Epithelial-Endothelial Interactions in the Developing Lung

(VEGF)

(Lammert et al, 2003)

Decreased Lung VEGF Expression in Human Infants with BPD

(Non-BPD)

(BPD)

(Bhat et al, 2000)

Neonatal VEGF Receptor Inhibition Causes Pulmonary Hypertension and Emphysema in Infant Rats

(Control)

(SU5416)

VEGF Receptor Inhibition Impairs Lung Vascular Growth in Neonatal Rats

(Control)
**VEGF Receptor Inhibition Disrupts Alveolar and Vascular Growth in the Infant Rat**

**Serial Changes in Lung eNOS Protein Content After SU5416 Injection**

(*p< 0.05)

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**Prolonged Inhaled NO Therapy Improves Lung Vascular Growth After VEGF Inhibition**

**Inhaled NO Inhibits Endothelial Cell Apoptosis After VEGFR Inhibition**

(Jen Ruey Tang)
Inhaled NO Improves Alveolarization in Experimental BPD in Preterm Lambs

Abnormal Lung Structure in Lamb BPD

Radial Alveolar Count

(Bland RD, 2005)

Endothelial Progenitor Cells (EPC) in Lung Vascular Development

- Circulating highly proliferative cells (CD34+) differentiate into endothelial cells (Asahara, 1997)
- Bone marrow cells (CD133+/VEGFR-2+) differentiate into endothelial cells (Reyes, 2002)
- EPCs isolated from cord blood (Ingram & Yoder, 2004)
- Roles during lung vascular development or the response to injury is unknown.

EPCs are Increased in Preterm Cord Blood

Hyperoxia (40% O₂) Decreases Preterm EPC Proliferation
Hyperoxia Reduces Lung and Bone Marrow Endothelial Progenitor Cells* in Neonatal Mice

Room Air  Hyperoxia (0.80)

Lung  Bone Marrow

(* CD45dim-; Sca-1+, CD133+, KDR+)

Study Question:
Can treatment with bone marrow-derived angiogenic cells (BMDAC) restore infant lung structure after neonatal hyperoxia?

Study Design:
BM-derived Tie2 GFP cells* (1 x 10^5 cells, RV injection)

BM -derived Angiogenic Cells Improve Lung Structure after Neonatal Hyperoxia

Neonatal hyperoxia w/ RA recovery  Neonatal hyperoxia w/ RA recovery + BMDAC

Co-localization Studies for Endothelial and Type II Cells After BMDAC Infusion

Endothelial  Type II Cell

Red = Factor VIII  Yellow = co-staining
Red = proSPC  Yellow = co-staining
Interim Summary: Impaired Angiogenesis in BPD

- Pulmonary hypertension in the developing lung impairs lung vascular growth and reduces alveolarization;
- Disruption of angiogenesis decreases alveolarization;
- Preserving endothelial cell survival and function or EPC treatment may improve lung structure in experimental BPD.

Early Inhaled NO Treatment of Premature Newborns with Respiratory Failure

Entire Study Population

(N = 587 patients)

Effects of Inhaled NO on Evolving BPD in Preterm Infants

Conclusions

• Disruption of vascular growth contributes to impaired lung structure and pulmonary hypertension in experimental BPD;
• Although inhaled NO improves lung structure in preclinical studies, its efficacy for the prevention of BPD remains uncertain.
• Therapeutic strategies that preserve endothelial function and survival may enhance lung vascular and alveolar growth in premature infants.
Inhaled NO Inhibits Endothelial Apoptosis after VEGFR Inhibition

Can Inhaled NO Prevent BPD?

- Potential therapeutic targets:
  - improve gas exchange, reduce FiO
  - lower pulmonary artery pressure
  - anti-inflammatory effects
  - antioxidant effects
  - sustain surfactant function/production
  - preserve or stimulate angiogenesis and alveolarization in the developing lung.
Inhibitors of Angiogenesis Decrease Lung Weight and Alveolarization in Infant Rats

**Lung:Body Weight Ratio**

![Graph showing lung:body weight ratio comparison between control, Fumagillin, and Thalidomide groups.](Jakkula et al, 2000)

**Radial Alveolar Counts**

![Graph showing radial alveolar counts comparison between control, Fumagillin, and Thalidomide groups.](Jakkula et al, 2000)

Chronic Intrauterine Pulmonary Hypertension Decreases Vessel Density in Fetal Sheep

**Vessel density**

![Graph showing vessel density comparison between control and PPHN groups.](Theresa Grover)

Pulmonary Hypertension Decreases VEGF and eNOS Expression in Fetal PAEC

**VEGF**

Normal PPHN

![Blot showing VEGF protein expression in Normal and PPHN groups.](Jason Gien)

**eNOS**

Normal PPHN

![Blot showing eNOS protein expression in Normal and PPHN groups.](Jason Gien)

Pulmonary Hypertension Decreases Endothelial Growth and Tube Formation *in vitro*

**Growth**

![Graph showing endothelial growth comparison between Normal and PPHN groups.](Jason Gien)

**Tube Formation**

![Graph showing tube formation comparison between Normal and PPHN groups.](Jason Gien)
VEGF Inhibition Causes PPHN

Inhibitors of Angiogenesis Decrease Lung Weight and Alveolarization in Infant Rats

Antiangiogenesis Agents Decrease Alveolarization

VEGF Receptor Inhibition Decreases eNOS Expression and Disrupts Lung Architecture in Fetal Rat Lung Explants
Mild Neonatal Hypoxia (FiO₂, 0.16) Impairs Distal Lung Growth in eNOS -/- Mice

Wild Type  eNOS -/-

(Balasubramaniam, 2003)

rhVEGF Treatment Enhances Lung Branching in eNOS-/- Mice

Pulmonary Hypertension Decreases Endothelial Growth and Tube Formation in vitro

Pulmonary Hypertension Decreases VEGF and eNOS Expression in Fetal PAEC

(Jason Gien)

(Jason Gien)
Preterm EPCs Proliferate More Rapidly than Term EPCs

Lung Pathophysiology of BPD

Central Airways
- Tracheomalacia
- Subglottic stenosis, cyst
- Granulomas
- Bronchomalacia
- Bronchial stenosis

Small Airways
- Structural remodeling
  - Mucus gland hyperplasia
  - Epithelial injury, edema
- Smooth muscle, fibrosis
- Bronchoconstriction
- Hyperreactivity

Distal Airspace and Vasculature:
- Decreased alveolarization, vascular growth
- Abnormal vascular remodeling, tone and reactivity
- Impaired lymphatic function, structure

Severe Bronchopulmonary Dysplasia

Age 6 months
Age 14 months
Age 23 months

(Chris Baker)

(Rob Castile)