Intracellular signaling and the development of Bronchopulmonary Dysplasia (BPD)

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Neonatal and Childhood Pulmonary Vascular Disease

Bronchopulmonary dysplasia:

- **Definition**
  - Is a chronic pulmonary interstitial and vascular disease that affects prematurely born infants.
  - Associated with increased O$_2$ requirements, chronic airway disease, pulmonary hypertension, poor somatic growth, and developmental delay.
  - Diagnosed in prematurely born infants requiring O$_2$ supplementation at 36 weeks corrected postnatal gestational age (Shennan 1988).

- **Incidence**
  - Inversely proportional with gestational age (Stevenson 1998 and Stoll 2010).
  - In the US, the rate of BPD controlled for gestational age has not changed in recent years despite advances in neonatal care (Smith et al J Pediatr 2005).
  - Because of increased premature delivery rates, the incidence of BPD has increased in the US during the past 20 years (Branum 2002).
  - BPD accounts for the second largest expenditure of health care dollars spent on pediatric diseases.

BPD is a developmental lung disease:

- **Antenatal Lung Injury**
  - Preterm delivery
  - Lung Injury
  - Interruption of lung development

- **Cytokine Exposure**
  - Infection
  - Ventilation
  - Oxygen

- **Infection**
  -bronchi
  -bronchioli
  -alveolar ducts
  -alveoli

**Embryonic**: 0 - 7 wks
**Pseudo-glandular**: 7 - 17 wks
**Canalicular**: 17 - 27 wks
**Saccular / Alveolar**: > 27 wks

Human lung development:

- trachea
- bronchi
- terminal bronchioli
- respiratory
alveolar ducts
-alveoli

Human lung development and BPD:

Embryonic Pseudo-glandular Canalicular Saccular / Alveolar trachea bronchi bronchioli terminal respiratory bronchioli alveolar ducts alveoli

0 - 7 wks 7 - 17 wks 17-27 wks

Risk period for BPD

Control BPD Control BPD

PECAM-1 VEGF

αSMA (black stain)

Bhatt... Maniscalco Am J Respir Crit Care Med 2001; Thibeault... Ekekezie Ped Pulm 2003

Developmental stage of lung injury influences pulmonary development:

Model: Doxycyclin-inducible Clara cell Interleukin (IL)-1β,

DOX-mediated IL-1β induction periods:

IL-1β levels in fetal and newborn lungs:

Mouse lung development stage:

Backstrom...Bry Ped Res 2011

NO and cGMP signaling:

• Review the NO / cGMP signaling cascade in vascular smooth muscle cells.
• Present data indicating that NO and cGMP signaling are abnormal in the injured newborn lung.
• Discuss approaches employed to modulate NO and cGMP signaling in newborn lung disease models.
• Provide new details about the mechanisms that regulate nuclear cGMP signaling.
EC SMC

Neuronal NOS (I)
Inducible NOS (II)
Endothelial NOS (III)

Gene
NOS1
NOS2A, 2B, 2C
NOS3

Neuronal
Immune system
CV system
Endothelium

Ca\(^{2+}\) modulated
arginine
arginine + NO

NOS III is up-regulated during saccular phase of rat lung development:

RNA blot:

Immunoblot blot:


NOS III expression is decreased in premature lambs with lung injury:

NOS III IB:

Pulmonary Arteries
Airways

Newborn Control Chronic Vent
3-4 weeks

Newborn
Control
Chronic Vent

MacRitchie...Bland Am J Physiol 2001;281:L1011.

NOS III deficiency alters lung development:

WT
NOS III -/-

PECAM

Han...Stewart. Circ Res 2004
Balasubramaniam...Abman AJP 2003
• Arginine and citrulline plasma levels are low in PPHN (Pearson 2001).
• ARG II is increased in endothelial cells of lambs with shunt-induced vascular injury (Sharma 2009), patients with PAH (Xu 2004), and in isolated human PASMC with hypoxia (Chen 2009).
• Polyamines and proline promote SMC proliferation and extracellular matrix (collagen) synthesis (Marschner 2011).

sGC subunit expression is up-regulated during the saccular phase of rat lung development:

sGC mRNA

sGC mRNA - ISH

sGC enzyme activity

Bloch...de la Monte Am J Physiol 1997.

sGC subunit expression is decreased in the injured preterm lamb lung:

sGCα1/β1 IB:

sGCα1/β1 IR:

Bland...Dahl AJP 2003.
**Pulmonary sGC isoform and enzyme activity levels are decreased in sGCα1 knockout (KO) mouse pups:**

*Model: C57BL6 homozygous sGCα1-deficient mice (Buys 2008).*

**NO Signaling Enzyme Expression:**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>sGCα1 KO</th>
<th>WT</th>
<th>sGCα1 KO</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>sGCα1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>sGCβ1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>sGCα2</td>
<td></td>
<td></td>
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<tr>
<td>PKGI</td>
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</tbody>
</table>

*α-tubulin: loading control.*

Representative immunoblot. sGCβ1 is decreased by >75% in KO pup lungs. N = 5–6 pups, P < 0.05.

**sGC Enzyme Activity:**

NO donor + sodium nitroprusside stimulation. N = 12 pups, *P < 0.05.*

**Decreased sGC activity is associated with diminished lung development:**

- P3
- P13 – 10 days of air or 70% O₂

**LV / Weight (ml/g):**

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>70% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>sGCα1 KO</td>
<td>0.10</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Septae / (200 µm):**

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>70% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>sGCα1 KO</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Scale bar 80 µm, *P ≤ 0.05; Bachiller...Roberts PAS 2013

**PKGI** exists in two isoforms, PKGIα and PKGIβ.

- NH₂-terminal PKGI contains auto-inhibitory (AI) pseudo substrate domain that binds with the catalytic region substrate recognition domain (SR) and thereby inhibits kinase activity.
- PKGI isoforms homodimerize or bind to cytosolic anchoring proteins.
- cGMP binding causes relaxation of PKGI, disruption of AI-SR interaction, and PKGI kinase activation.

**NO**

PDE, phosphodiesterase; CNG, cyclic nucleotide-gated ion channels
NO / cGMP stimulates PKGI phosphorylation of cytoplasmic targets that cause vasodilatation:

- MLCP/MBS: myosin-binding subunit of myosin light chain phosphatase
- RGS-2: regulator of G-protein substrates - 2
- IP3 receptor associated cGMP kinase substrate
- TFII-I: a multifunctional transcriptional regulator

NO / cGMP signal targeting increases PKGI activity in several newborn lung injury models:


PKGI modulates nuclear signaling through incompletely understood mechanisms:

- NO / cGMP regulate SMC proliferation (Garg 1989).
- PKGI modulates how NO / cGMP regulate PA and AoSMC phenotype (Chiche 1998).
- PKGI regulates vascular SMC phenotype (Boerth 1997, Zhou 2007).
- cGMP stimulates nuclear PKGI translocation and CREB phosphorylation (Pilz 1996 - 2002).
- PKGI does not localize to the nucleus or regulate gene expression (Collins 1999, Browning 2001, Fell 2002).

PKGI post-translational modifications regulate nuclear PKGI localization:

- * PKGIγ is ~ 60 kDa kinase fragment of PKGI

**PKGI proteolysis is required for nuclear cGMP signaling in SMC:**

A Propensity proteolysis site:

B MAb1 MAb2 MAb3 MAb4 MAb5

C PKGIα PKGIβ PKGIγ

D PKGIα PKGIβ PKGIγ

*Sugihara...Roberts Circ Res 2008*

**Proprotein convertases (PCs) cleave PKGI and regulate nuclear PKGIγ localization:**

- PCs are a family of 10 proteases that cleaving pre-proteins and thereby regulating protein function.
- Furin, PC5, and PC7 are expressed in vascular SMC.

A Anti-PKGI

B Anti-PKGI

C Anti-PKGI

**PKGI co-localizes with the TGN38 and furin in the Golgi apparatus:**

A PKGI TGN38

**PKGI transport to GA regulates PKGI proteolysis and nuclear PKGIγ translocation:**

A IRAG PKGIβ

B IRAG PKGIβ

C PKGIβ

*Sugihara...Roberts Circ Res 2008*

**PKGI transport to GA regulates PKGI proteolysis and nuclear PKGIγ translocation:**

A IRAG PKGIβ

B IRAG PKGIβ

C PKGIβ

*Abbreviations: ER-GIC, ER-Golgi intermediate compartment; TGN, trans-Golgi network

*Sugihara...Roberts Circ Res 2008*
Golgi apparatus disruption inhibits PKGI proteolysis and nuclear PKGIγ localization:

**BFA**: Brefeldin A; **NZ**: nocodazole

GTP

NO

sGCβ

sGCα

EC SMC

Cytosolic targets

Vasodilatation

PKGI γ

arginine

NOS

O2

cGMP

PKGI

PCs

PKGI

TGN

GA

ER-GIC

ER

Abbreviations: ER-GIC, ER-golgi intermediate compartment; TGN, trans-Golgi network

Working model of mechanisms that regulate nuclear PKGI signaling:

Summary

- BPD is a developmental lung disease associated with injury during the saccular and alveolar phases of lung development.
- Several growth factor and intracellular signaling systems are disrupted in the injured newborn lung.
- Nitric oxide and cGMP signaling is decreased in newborn lung injury and might contribute to disruption of normal pulmonary development.
- cGMP-dependent protein kinase I regulates cytoskeletal kinetics and cell phenotype, although the effect of PKGI on lung development and of PKGI post-translational modifications on nuclear signaling are incompletely understood.